AL/OE-TR-1994-0119
NMRI-94-111
WRAIR/TR-94-0009

ARMSTRONG LABORATORY

1993 TOXIC HAZARDS RESEARCH UNIT ANNUAL REPORT

D. E. Dodd
P. M. (Fleenor) Smith

MANTECH ENVIRONMENTAL
TECHNOLOGY, INC.
PO BOX 31009
DAYTON, OH 45437-0009

October 1994

FINAL REPORT FOR THE PERIOD 1 OCT 1992 THROUGH 30 SEPT 1993

AIR FORCE MATERIEL COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO 45433-6573

NOTICES

When US Government drawings, specifications or other data are used for any purpose other than a definitely related Government procurement operation, the Government thereby incurs no responsibility nor any obligation whatsoever, and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

Please do not request copies of this report from the Armstrong Laboratory. Additional copies may be purchased from:

National Technical Information Service
5285 Port Royal Road
Springfield, Virginia 22161

Federal Government agencies and their contractors registered with the Defense Technical Information Center should direct requests for copies of this report to:

Defense Technical Information Center
Cameron Station
Alexandria, Virginia 22314

DISCLAIMER

This Technical Report is published as received and has not been edited by the Technical Editing Staff of the Armstrong Laboratory.

TECHNICAL REVIEW AND APPROVAL

AL/OE-TR-1994-0119
NMRI-94-111
WRAIR/TR-94-0009

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

TERRY A. CHILDRESS, Lt Col, USAF, BSC
Director, Toxicology Division
Armstrong Laboratory

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave Blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 1994	Final -01 October 1992 - 30 September 1993

4. TITLE AND SUBTITLE
1993 Toxic Hazards Research Unit Annual Report

5. FUNDING NUMBERS
Contract F33615-90-C-0532
PE 62202F
PR 6302
TA 630200
WU 63020002

6. AUTHOR(S)
D.E. Dodd and P.M. (Fleenor) Smith (Editors)

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
ManTech Environmental Technology, Inc.
P.O. Box 31009
Dayton, OH 45437-0009

8. PERFORMING ORGANIZATION REPORT NUMBER
AR-4300-93J

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Armstrong Laboratory, Occupational and Environmental Health Directorate
Toxicology Division, Human Systems Center
Air Force Materiel Command
Wright-Patterson AFB OH 45433-7400

10. SPONSORING;MONITORING AGENCY REPORT NUMBER
AL/OE-TR-1994-0119
NMRI-94-111
WRAIR/TR-94-0009

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

This report presents a review of the activities of the Toxic Hazards Research Unit (THRU) for the period 01 October 1992 through 30 September 1993. The THRU conducts descriptive, mechanistic, and predictive toxicology research and toxicological risk assessments to provide data to predict health hazards and to assess health risks associated with human exposure to chemicals and materials associated with military systems and operational environments. The report includes summaries of ongoing or completed research activities for the individual toxicology research requirements of the U.S. Air Force, Army, and Navy; highlights of the research support elements and conference activities of the THRU; and appendices that describe the THRU organization and its publications and presentations. The majority of the report describes the progress attained in toxicological studies on a wide variety of chemicals and materials to include tetrachloroethylene, iodotrifluoromethane, ammonium dinitramide, HCFC-123, trichloroethylene, toluene, liquid propellant formulation 1846, vinyl chloride and trichloroethylene mixture, methylene chloride, MIL-H-19457C hydraulic fluid, acrolein and acrolein/Syloid 244 mixture, 1,3,3-trinitroazetidine, and 1,3,5-trinitrobenzene. The THRU also conducted research on lactational transfer and dermal absorption of chemicals, on toxicological risk assessment methods, and evaluated statistical methods for assessing military populations as a subgroup of the public at-large.

14. SUBJECT TERMS
Genotoxicity; Lactational Transfer; Dusty Mustard Surrogate; Risk Assessment; Reproductive Toxicity; Screening Information Data Set (SIDS); Physiologically Based Pharmacokinetic (PBPK) Modeling

15. NUMBER OF PAGES
230

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

THIS PAGE INTENTIONALLY LEFT BLANK

PREFACE

The 30th Annual Report of the Toxic Hazards Research Unit (THRU) presents research and research support efforts conducted by ManTech Environmental Technology, Inc. on behalf of the U.S. Air Force, the U.S. Army, and the U.S. Navy under Department of the Air Force Contract No F33615-90-C-0532. This document represents the third annual report for the current THRU contract and describes accomplishments from 01 October 1992 through 30 September 1993.

Operation of the THRU under this contract was initiated on 16 January 1991 under Project No. 6302, "Occupational and Environmental Toxic Hazards in Air Force Operations," Work Unit No. 63020002, "Toxic Hazards Research." This research effort is cosponsored by the Army Medical Research Detachment, Walter Reed Army Institute of Research (WRAIR), Work Unit Nos. 611102.S15L and 612787.878L, and by the Naval Medical Research Institute Detachment/Toxicology (NMRI/TD), Work Unit No. M0096.004.0006, "Criteria for Exposure Limits in Navy Operational Environments."

The Toxicology Division, Occupational and Environmental Health Directorate, Armstrong Laboratory (AL/OET), Wright-Patterson Air Force Base, Ohio, provided the technical direction for this contract. Lt Col Terry A. Childress, Director of the Toxicology Division, served as the Contract Technical Monitor. That portion of the work effort sponsored by the Army was under the direction of LTC Roland E. Langford, Detachment Commander, and LTC Daniel J. Caldwell, Senior Scientist of the Medical Research Detachment. That portion of the work effort sponsored by the Navy was under the direction of the NMRI/TD Officer-in-Charge, CAPT David A. Macys, MSC, USN. Darol E. Dodd, Ph.D., served as the ManTech Environmental THRU Program Manager.

The contents and the preparation of this report represent the combined efforts of the ManTech Environmental staff of the THRU and the staff of the ManTech Environmental Technical Publications and Graphics Department. Acknowledgement is made to Ms. JoAnne Barker, Ms. Shelia Brooks, Ms. Shelia Elliott, Ms. Cindy Matthews,, and Mr. Pete Winz for their assistance in the preparation of this report.

TABLE OF CONTENTS

TABLE OF CONTENTS

SECTION 1

ABBREVIATIONS

A	Absorbance units
ACGIH	American Council of Governmental Industrial Hygienists
ADN	Ammonium dinitramide
AFB	Air Force Base
AFOSH	Air Force Occupational Safety and Health
AL/OET	Toxicology Division, Occupational and Environmental Health Directorate, Armstrong Laboratory
AL/OEVM	Armstrong Laboratory, Comparative Medicine Branch
ALD	Approximate lethal dose
ALKP	Alkaline phosphatase
ALT	Alanine aminotransferase
AN	Ammonium nitrate
AST	Aspartate aminotransferase
BEI	Biological exposure indices
BUDs	Basic Underwater Demolition (team)
CDFE	2-Chloro-1,1-difluoroethylene
CF_3I	Iodotrifluoromethane (trifluoroiodomethane)
CHO	Chinese hamster ovary
CMAT	Concentration in milk
CO	Carbon monoxide
CO_2	Carbon dioxide
DCA	Dichloroacetic acid
DMC	Deuterated methylene chloride analog
DMSO	Dimethylsulfoxide
DNB	Dinitrobenzene
EC_{50}	Medium effective concentration
ECRI	Excess cancer risk estimate for infants
EMH	Extramedullary hematopoiesis
EPA	U.S. Environmental Protection Agency

F-344	Fisher 344 (rats)
FID	Flame ionizing detector
GC	Gas chromatograph(y)
GLP	Good Laboratory Practice
GLPS	Good Laboratory Practice Standards
GSH	Glutathione
GST	Glutathione transferase
HAN	Hydroxylammonium nitrate
HbCO	Carboxyhemoglobin
HCFC-123	2,2-Dichloro-1,1,1-trifluoroethane
HCFC-133a	2-Chloro-1,1,1-trifluoroethane
HD	Mustard gas
HGPRT	Hypoxanthine-guanine phosphoribosyl-transferase
HMX	Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
HPLC	High performance liquid chromatograph(y)
H_2SO_4	Sulfuric acid
HSR	Health and Safety Representative
IDM	Infant dose from milk
IRBC	Indoor residential background concentration
KCl	Potassium chloride
LAN	Local area network
LD_{50}	Median lethal dose
MBW	Mean body weight
MC	Methylene chloride
MFO	Mixed function oxidase
MRC	Midwest Regional Chapter (Society for Quality Assurance)
MS	Mass spectrometry
MSDS	Material Safety Data Sheet
NADPH	Reduced nicotinamide-adenine dinucleotide phosphate
NMRI/TD	Naval Medical Research Institute Detachment/Toxicology
NPSH	Nonprotein sulfhydryl
NYDH	New York State Department of Health

NZW	New Zealand White (rabbits)
OSHA	Occupational Safety and Health Administration
PBPK	Physiologically based pharmacokinetic
PC	Personal computer
PCE	Perchloroethylene (tetrachloroethylene)
PCEs	Polychromatophilic erythrocytes
QA	Quality assurance
RCRA	Resource Conservation and Recovery Act
RO	Reverse osmosis
SD	Standard deviation
SEAL	SEa, Air, Land (team)
SEM	Standard error of the mean
SIDS	Screening Information Data Set
SOP	Standard operating procedure
SQA	Society of Quality Assurance
T_4	Thyroxine
TBG	Thyroxine-binding globulin
TBP	Tributylphenyl phosphate
TCA	Trichloroacetate
TCA	Trichloroacetic acid
TCE	Trichloroethylene
TCOH	Trichloroethanol
TCP	Tricresyl phosphate
TCRI	Total cancer risk estimate for infants
tDCE	*trans*-1,2-Dichloroethylene
TEAN	Triethanolammonium nitrate
TFA	Trifluoroacetic acid
TGA	Thermo gravimetric analyzer
THRU	Toxic Hazards Research Unit
TLV	Threshold limit value
Tmet P	Total lung metabolism
TNAZ	1,3,3-Trinitroazetidine

TNB	1,3,5-Trinitrobenzene
TOTINF	Total daily infant dose
TRANS	*trans*-1,2-Dichloroethylene
VC	Vinyl chloride
WPAFB	Wright-Patterson Air Force Base
XM46	Liquid Propellant formulation 1846

SECTION 2
INTRODUCTION

This report presents a review of the activities of the ManTech Environmental Technology, Inc., Toxic Hazards Research Unit (THRU), for the period 01 October 1992 through 30 September 1993. ManTech Environmental's THRU is an on-site, contractor-operated, United States Air Force, Army, and Navy multidisciplinary research program. The THRU conducts descriptive, mechanistic, and predictive toxicology studies and toxicological risk assessments to provide data to predict health hazards and to assess the health risks associated with human exposure to chemicals and chemical materials of interest to the military. The major goal of the THRU's research efforts is to contribute to safe military operations, including safe occupational and environmental conditions. An additional goal of the THRU is to advance the state-of-the-art in toxicology research and risk assessment techniques.

In accordance with the THRU contract's Statement of Work and specific technical directives (study requests) provided by the Contract Technical Monitor, the THRU also coordinates toxicology conferences, expert workshops, and program reviews. Research support benefitting both THRU and government research efforts is provided in the areas of special test equipment design, fabrication, validation, modification, and maintenance; mathematics and biometry; computer systems management and programming; necropsy and histology techniques; toxicology information databases and library management; quality assurance; health and safety; and documentation and report preparation. The THRU's research support and administrative elements are integral to the quality, continuity, and productivity of its scientific research efforts.

The THRU conducts research on a variety of materials that may range from pure chemicals to poorly defined mixtures. They include, but are not limited to fuels, lubricants, solvents, additives, components of explosives, propellants, paints, solvents, structural materials, training agents, and combustion products. Descriptive toxicology is used to identify toxic effects, target organs, and dose–response effects associated with different exposure routes, concentrations, and durations. Mechanistic toxicology is performed to determine toxicokinetics, mechanisms of action, and dynamics of expression of the toxic effects of the material of interest. Predictive toxicology involves the development, validation, and application of computer simulation models to describe quantitative dose–response relationships based on quantified input parameters such as exposure concentration, partition coefficients, respiratory rate, blood flows, rate of metabolite formation, rate of chemical excretion, and metabolic enzyme constants. These models are used to define target organ toxicity based on the tissue-specific dose and are used in intra- and interspecies extrapolation. Data generated via descriptive, mechanistic, and predictive toxicology studies are used together with interpreted literature data

to produce toxicological risk assessments that contribute to safe military systems as well as safe occupational and environmental conditions.

The research and support efforts of the THRU represent a continuum of activities that may overlap two or more years depending upon the study scheduling and the extent of the research that is required. During this reporting period, studies performed in response to requirements of the Air Force included physiologically based pharmacokinetic modeling of perchloroethylene, HCFC-123, and mixtures of vinyl chloride and trichloroethylene; perchloroethylene exposure and risk assessments; determination of blood concentrations following dermal exposure to chemical vapors; analysis of metabolites of trichloroethylene; genetic toxicity of 1,3,3-trinitroazetidine; acute toxicity studies of ammonium dinitramide and iodotrifluoromethane; and generation and analysis of toluene exposures.

During this reporting period, the THRU received several study requests for toxicology research in support of the Army. The THRU initiated studies to evaluate the reproductive toxicity potential of 1,3,5-trinitrobenzene and Liquid Propellant Formulation 1846 (XM46). Additionally, quality assurance support was provided for the teratologic evaluation of XM46 on a study performed by Air Force personnel.

Toxic Hazards Research Unit studies conducted to support the Navy included continuation of a statistical methods study to define techniques for assessing the variability in sensitivity of the human population and subpopulations in order to develop a basis for logically altering safety factors used in risk assessments applicable to military populations, evaluation of the endocrine effects of MIL-H-19457C hydraulic fluid, evaluation of the lung response to acrolein and acrolein/Syloid 244 mixtures (dusty mustard surrogates), development and evaluation of methods for assessing the insult of combustion products, and analysis of the metabolism of methylene chloride in the mouse and its implications for human carcinogenic risk.

During this reporting period, the THRU provided work effort in support of three toxicology conferences that are part of the series of annual toxicology conferences that have been coordinated by the THRU since 1965. The proceedings of the 1992 toxicology conference "Applications of Advances in Toxicology to Risk Assessment" were compiled by the THRU and distributed as a peer-reviewed publication by the journal *Toxicology Letters* (Vol. 68, 1,2, 1993). Additionally, the full proceedings of the conference, including poster abstracts, were compiled for publication as an Air Force Technical Report (AL/OE-TR-1993-0059, NMRI-93-18). The 1993 toxicology conference "The Risk Assessment Paradigm After Ten Years: Science, Policy, and Practice Then, Now, and in the Future" was conducted in May at the Hope Hotel and Conference Center at Wright-Patterson Air Force Base. The conference included six general sessions and a poster session. The proceedings of this conference have been compiled by the THRU for publication as a peer-reviewed special issue of the journal *Risk Analysis*, and the full proceedings are being compiled for

publication as an Air Force Technical Report. In August 1993, the THRU initiated its planning efforts for the 1994 toxicology conference, "Temporal Aspects in Risk Assessment for Noncancer Endpoints."

The execution of the THRU contract Statement of Work involves the integrated effort of a multidisciplinary staff of scientists, research technicians, research support, and administrative personnel. Sections 3, 4, and 5 of this report emphasize the technical activities of the THRU. Sections 6 and 7 present highlights of the conferences and research support activities, including research engineering, mathematics and statistics, pathology support, computer and electronic support, quality assurance, and health and safety. Section 8 of this report is a set of appendices that describe the THRU organization, its personnel, and its publications and presentations.

Historically, the THRU has prepared annual reports on its research efforts. In general, these annual reports present summaries or highlights of the technical projects (study requests) that were directed by the Air Force, Army, and Navy. More descriptive reports on the THRU's research activities are prepared upon completion of individual study requests and are published as technical reports. Technical reports also are prepared following the conferences and most workshops coordinated by the THRU. Copies of these technical reports are available from the National Technical Information Service or the Defense Technical Information Center.

3.1 PHYSIOLOGICALLY BASED SIMULATION OF PERCHLOROETHYLENE (PCE) PHARMACOKINETICS IN HUMANS

J.M. Gearhart[1], D.A. Mahle, C.S. Seckel, and C.D. Flemming

ABSTRACT

Perchloroethylene (tetrachloroethylene, PCE) is a commercially important solvent used in dry cleaning and as a degreasing agent that commonly occurs as a ground water contaminant. To assist in the determination of the potential risk to humans exposed to PCE, a physiologically based pharmacokinetic (PBPK) model describing the kinetics of PCE in humans was developed and used to simulate a number of different human exposure data sets found in the literature. Mean values and error estimates for human blood/air and tissue/air partition coefficients were determined in the laboratory. Physiological parameters for humans, including estimates of parameter variability, were obtained from the physiological literature or from the published PCE kinetic data. A classical pharmacokinetic model describing trichloroacetate (TCA) pharmacokinetics was combined with the PBPK model of PCE to provide for a method of estimating the amount of PCE metabolized to TCA. Simulation of PCE in exhaled air and blood provided good estimates of the experimental data. The model simulations of blood and urinary levels of TCA provided reliable estimates of the laboratory data and confirmed the very low level of PCE metabolism in humans. Human metabolism was described with an apparent $V_{max} \approx$ 0.6 mg/h and $K_m \approx$ 7.7 mg/L. Although most human data sets were adequately described by the PBPK model, some segments of the simulations deviated from the data, especially the exhaled breath washout phase after longer or higher inhalation exposures to PCE. Incorporation of Monte Carlo simulations utilizing measured parameter variability provided a range of model predictions with error estimates that encompassed the actual human exposure data.

INTRODUCTION

Physiologically based pharmacokinetic (PBPK) models have become useful tools in the risk assessment process for deriving internal dose estimates from exposure to chemicals by integrating information on the administered dose, the physiological structure of the mammalian species, and the biochemical properties of the specific chemicals. In order for these models to find their application in determining excess risk from exposure to a chemical, the variability of the model parameters used in describing the pharmacokinetic data should be incorporated into the overall simulation of the pharmacokinetic data. In this study, the

---

[1]Current address: K.S. Crump Group, ICF Kaiser International, Ruston, LA.

interindividual variability of blood/air and tissue/air partition coefficients was determined experimentally for humans and then was combined with the published variation of body organ volumes and flow rates in a PBPK model for perchloroethylene (tetrachloroethylene, PCE) and a one-compartment model for trichloroacetate (TCA).

MATERIALS AND METHODS

Partition Coefficients

Human blood/air and tissue/air partition coefficients for PCE were determined as described by Gargas *et al.* (1989). Heparinized human blood samples (1 mL) were pipetted into 25 mL liquid scintillation vials and then a gas-tight syringe was used to add 1 mL of PCE from a standard bag to each vial. The vial was then heated at 37 °C with shaking for 3 h. The amount of PCE in the vial head space was then determined by gas chromatography with flame ionization detection. Human tissues obtained from a cadaver were homogenized and 50- to 100-mg samples were smeared on the wall of the sample vials and then were treated as in the blood sample determination.

Multiple blood samples (n=7) were determined for nine different individuals. Analysis of variance was utilized to determine the inter- versus intraindividual variation for the blood/air partition coefficients.

PBPK Model Structure

A five-compartment PBPK model (Figure 3.1-1) was used to describe the pharmacokinetics of PCE and a one-compartment pharmacokinetic model was used to describe TCA blood levels in humans exposed to PCE.

Monte Carlo Simulation

The means and standard deviations of each input parameter that was varied in the PBPK model (Table 3.1-1) were used as input parameters to the Monte Carlo simulation. The Monte Carlo method used was a variation of the Latin Hypercube. The simulations were accomplished using the software package PBPK_Sim (K.S. Crump Division/Clement IC, Ruston, LA). Postexposure measurements were made of PCE in expired breath and of PCE and TCA in blood from volunteers exposed for 4 h to 72 ppm PCE (Monster et al., 1979) and of PCE in expired breath from volunteers exposed for 8 h to 200 ppm PCE (Fernandez et al., 1976). These two data sets were used for model validation. Each data set was simulated using 300 simulations.

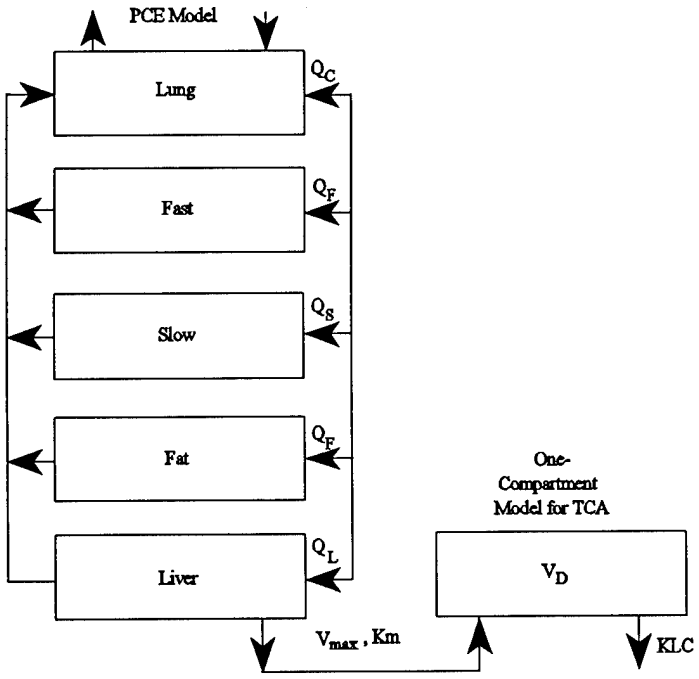


Figure 3.1-1. A PBPK Model for PCE, with a One-Compartment Model for TCA.

TABLE 3-1.1. PARAMETER TREATMENT FOR MONTE CARLO SIMULATION

Parameter	Mean	S.D.	Distribution/ Constrained	Upper Limit	Lower Limit
VMAXC	0.6	0.12	Lognormal/No		
KM	7.7	2.31	Lognormal/No		
FCTA	0.9	0.27	Lognormal/No		
KU	0.01	0.003	Lognormal/No		
VD	75.0	22.5	Lognormal/No		
QPC	24.0	3.74	Normal/Yes	50.0	5
QCC	16.5	1.58	Normal/Yes	50	5
QRC	0.52	0.11	Normal/Yes	0.7	0.01
QFC	0.05	0.01	Normal/Yes	0.7	0.01
QSC	0.24	0.048	Normal/Yes	0.7	0.01

TABLE 3-1.1. PARAMETER TREATMENT FOR MONTE CARLO SIMULATION

Parameter	Mean	S.D.	Distribution/ Constrained	Upper Limit	Lower Limit
QLC	0.19	0.037	Normal/Yes	0.7	0.01
BW	70.0	14.0	Normal/Yes	97	57
VSC	0.621	0.12	Normal/Yes	0.8	0.01
VFC	0.19	0.048	Normal/Yes	0.8	0.01
VLC	0.0314	0.0062	Normal/Yes	0.8	0.01
VRC	0.0371	0.0074	Normal/Yes	0.8	0.01
PF	125.2	18.8	Normal/Yes	250	2.5
PL	5.3	0.8	Normal/Yes	250	2.5
PS	6.1	0.9	Normal/Yes	250	2.5
PR	5.1	1	Normal/Yes	250	2.5
PB	11.6	2.3	Normal/Yes	23	5
PLM	0.25	0.08	Normal/Yes	1	0.1

RESULTS

Means and standard deviations of the measured human blood/air and tissue/blood partition coefficients are shown in Table 3.1-1. Measured concentrations of PCE in expired breath (Figure 3.1-2) and blood (Figure 3.1-3) and of TCA in blood (Figure 3.1-4) fell within the limits of the Monte Carlo simulations of human exposure to 72 ppm PCE for 4 h. Likewise, measured concentrations of PCE in expired breath (Figure 3.1-5) fell predominantly into the limits of the Monte Carlo simulations of human exposure to 200 ppm PCE for 8 h.

DISCUSSION

The variability of human blood/air partition coefficients was determined for PCE in the laboratory and then was combined with human physiological data from the literature to derive input parameter values for a PBPK simulation of controlled human exposures to PCE. For all exposure data sets, the 95% confidence interval output from the PBPK_Sim (Monte Carlo) simulations produced a range of curves that encompassed the mean data values for the different exposures. By varying PBPK model input parameters in accordance with the experimentally determined values for PCE and the human physiology, it was possible to provide a stochastic simulation of human inhalation exposures to PCE. The results demonstrate that the specified parameter uncertainty is consistent with the experimental data.

REFERENCES

Fernandez, J., E. Guberan, and J. Gaperos. 1976. Experimental human exposures to tetrachloroethylene vapor and elimination in breath after inhalation. *Am. Ind. Hyg. Assoc. J.* 37:143-150.

Gargas, M.L., M.E. Andersen, and H.J. Clewell III. 1989. Partition coefficients of low-molecular-weight volatile chemicals in various liquids and tissues. *Toxicol. Appl. Pharmacol.* 97:87-99.

Monster, A.C., G. Boersma, and H. Steenweg. 1979. Kinetics of tetrachloroethylene in volunteers; Influence of exposure concentration and work load. *Int. Arch. Occup. Environ. Health* 42:303-309.

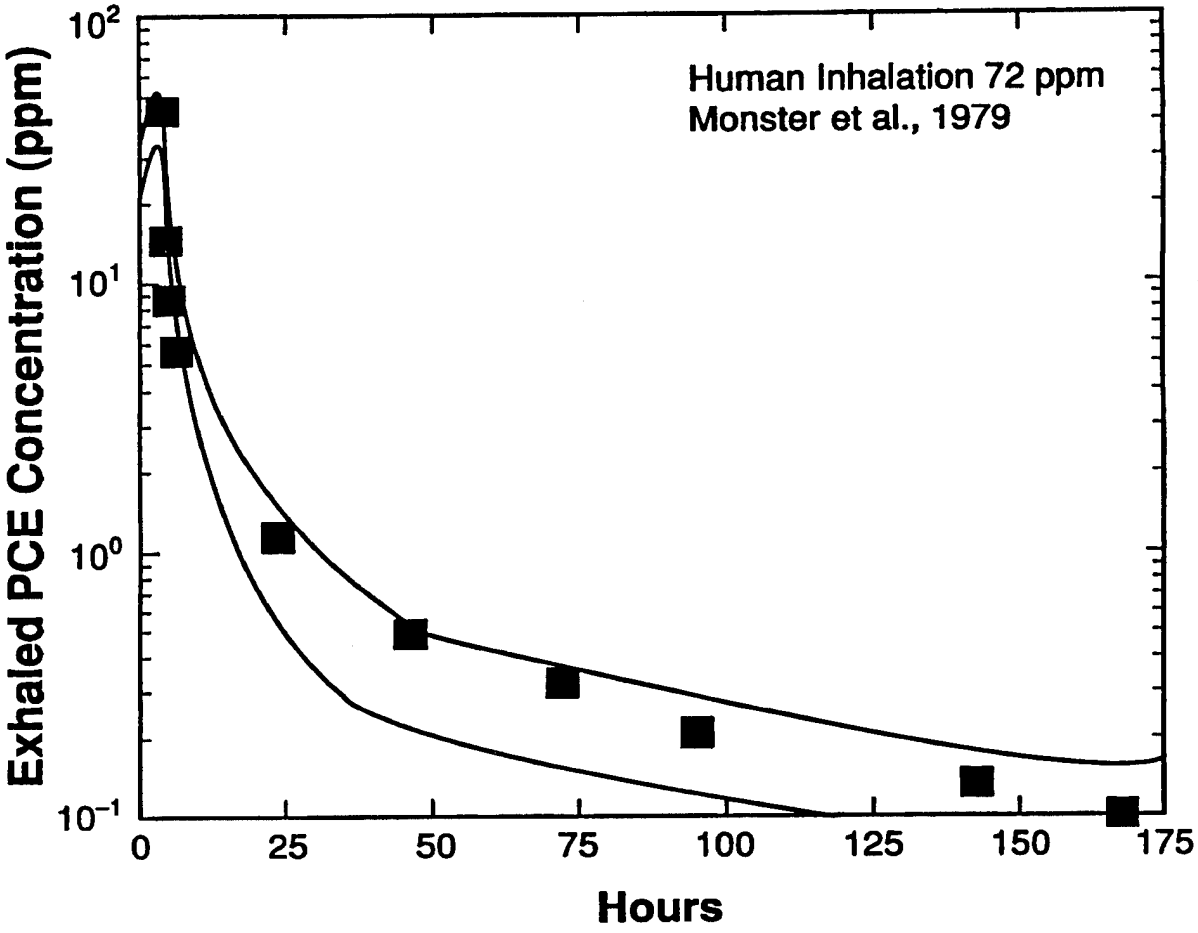


Figure 3.1-2. Exhaled PCE Concentration from Humans after an Inhalation Exposure to 72 ppm PCE. Data points represent the mean and the solid lines represent the 95% confidence limits of the highest and lowest Monte Carlo PBPK simulations.

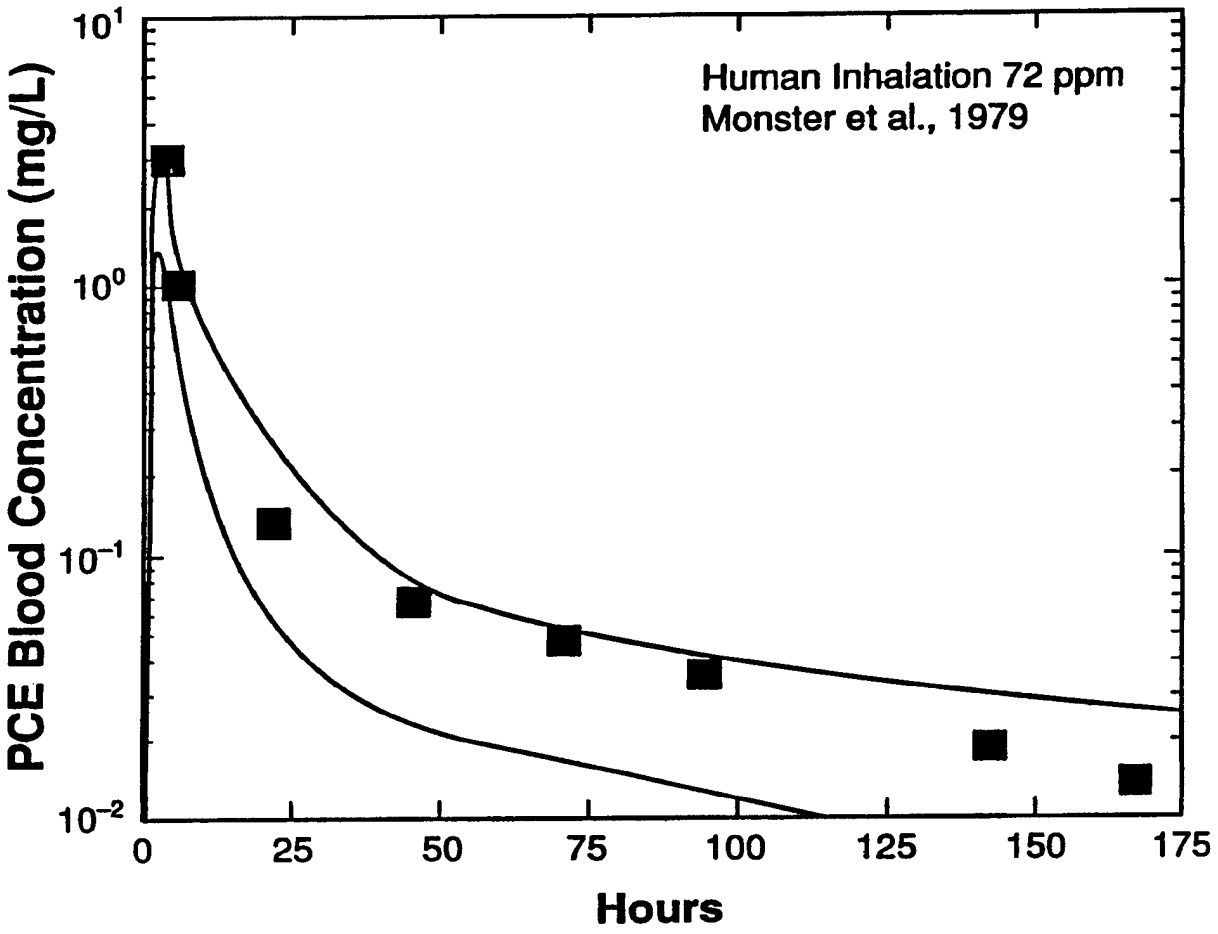


Figure 3.1-3. The PCE Blood Concentration from Humans after an Inhalation Exposure to 72 ppm PCE. Data points represent the mean and the solid lines represent the 95% confidence limits of the highest and lowest Monte Carlo PBPK simulations.

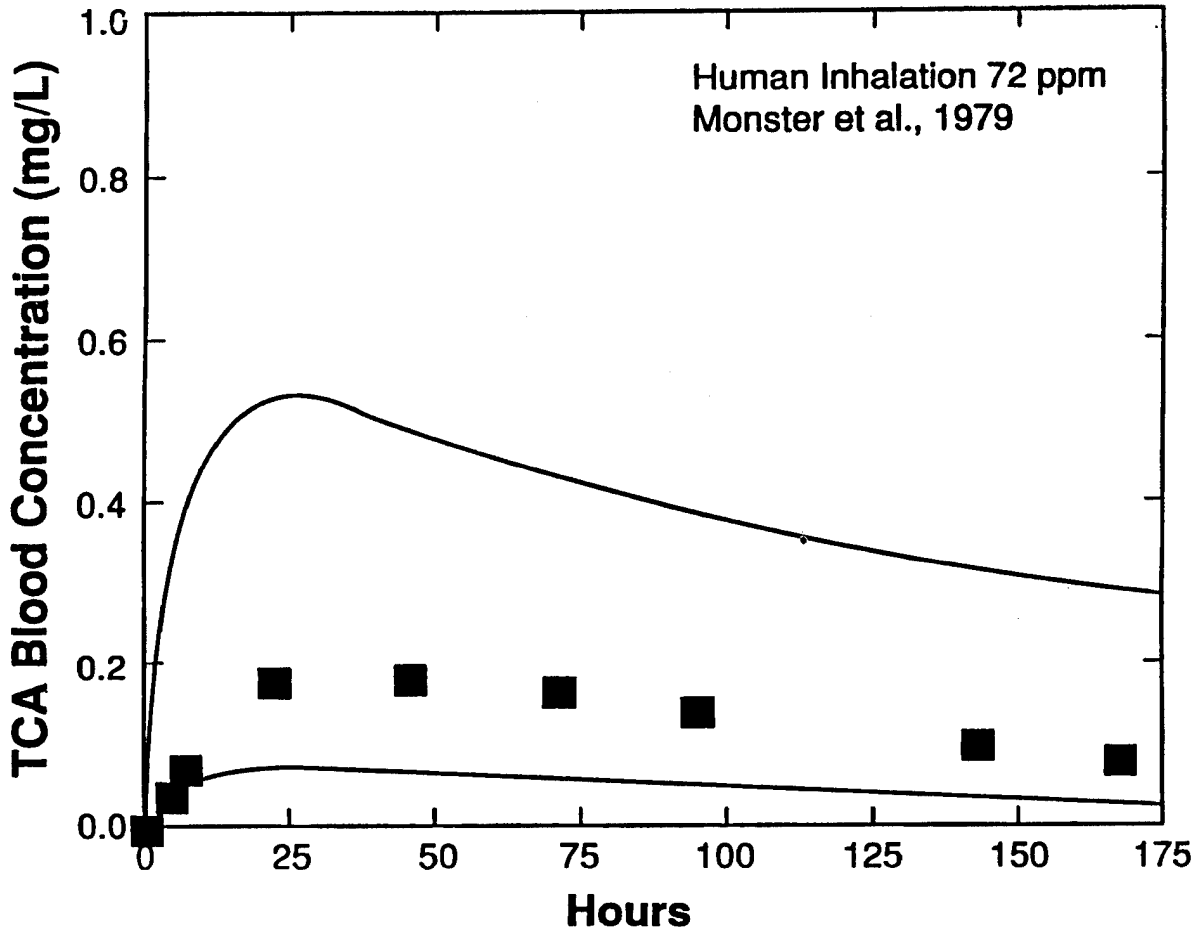


Figure 3.1-4. The TCA Blood Concentration from Humans after an Inhalation Exposure to 72 ppm PCE. Data points represent the mean and the solid lines represent the 95% confidence limits of the highest and lowest Monte Carlo PBPK simulations.

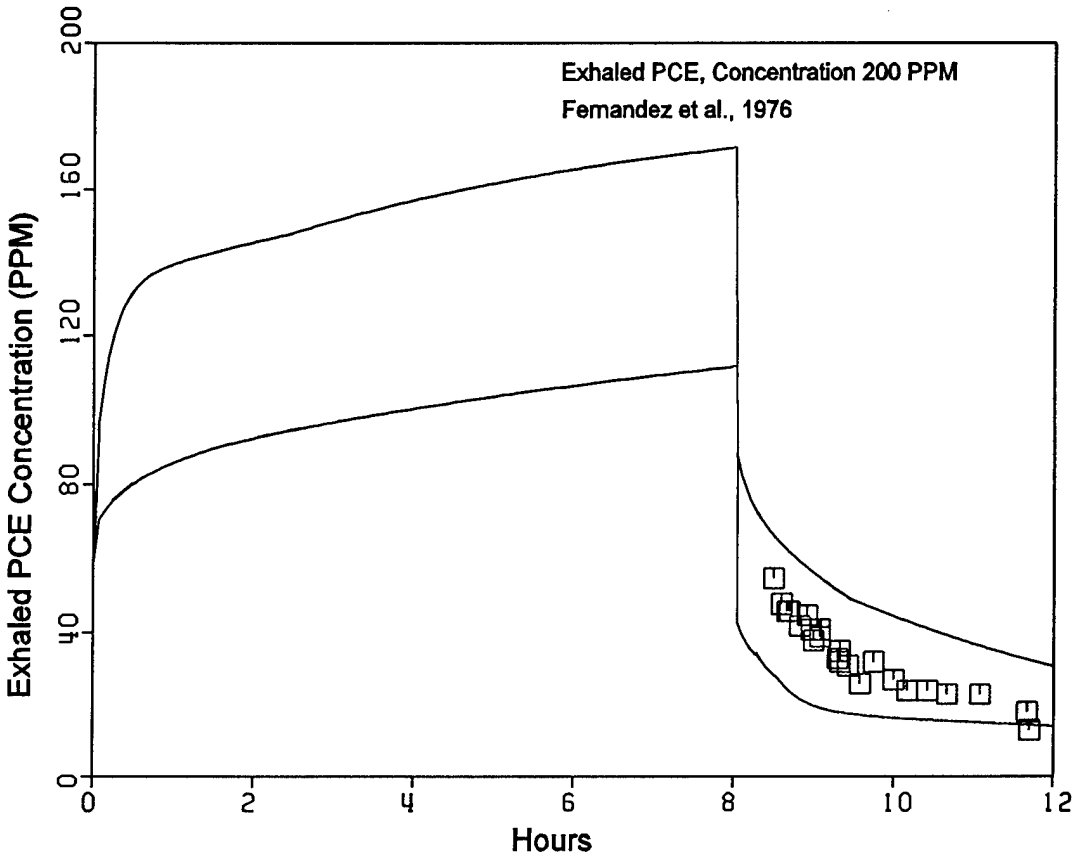


Figure 3.1-5. Exhaled PCE Concentration from Humans after an Inhalation Exposure to 72 ppm PCE. Data points represent the mean and the solid lines represent the 95% confidence limits of the highest and lowest Monte Carlo PBPK simulations.

3.2 DOSE-DEPENDENT METABOLISM OF 2,2-DICHLORO-1,1,1-TRIFLUOROETHANE (HCFC-123): A PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL IN THE MALE FISCHER 344 RAT

A. Vinegar, R.J. Williams[1], J.W. Fisher[1], and J.N. McDougal[1]

ABSTRACT

2,2-Dichloro-1,1,1-trifluoroethane (HCFC-123) is used industrially as a refrigerant, a foam blowing agent, and a solvent. It also is being considered as a replacement for Halons and chlorinated fluorocarbons, which have been banned by the Montreal Protocol because they deplete atmospheric ozone. HCFC-123 is a relatively nontoxic chemical that may cause anesthetic effects at high concentrations and several types of organ changes in chronic exposures. It is a fairly lipophilic chemical that is primarily oxidatively metabolized to trifluoroacetic acid (TFA) and eliminated in the urine. We exposed Fischer 344 rats to 1.0, 0.1, and 0.01% HCFC-123 by inhalation and measured blood, fat, and exhaled breath concentrations for the parent and blood and urine concentrations for the primary metabolite. A physiologically based pharmacokinetic (PBPK) model was developed that included a gut compartment and a variable size fat compartment in addition to the standard flow-limited compartments. Compartment volumes and flows were chosen from the literature, partition coefficients were measured in the laboratory, and metabolic parameters were optimized from experimental data using model simulations. Laboratory experiments showed that the TFA blood concentration during the 1.0% exposure was more than 50% less than the TFA blood concentration during the 0.1% exposure. After cessation of the 4-h exposure, TFA blood concentrations from the 1.0% exposure rebounded and peaked about 22 h after the exposure at about the same concentration as the 0.1% peak. This rebound phenomenon suggested that it was not killing of the metabolic enzymes but suppression that made the TFA blood concentrations lower than expected. A suppression constant for metabolism was added to the model based on the TFA concentrations and suggestions in the literature that a structurally similar chemical, halothane, exhibits the same behavior. The resulting PBPK model adequately simulated the entire set of pharmacokinetic data. This combination of laboratory experimentation and PBPK modeling can be applied to relate the levels of parent and metabolite to toxic effects with some hope of elucidating the toxic species. These results will improve extrapolations in dose, route, and species.

---

[1]Armstrong Laboratory, Toxicology Division, Wright-Patterson AFB, OH.

INTRODUCTION

2,2-Dichloro-1,1,1-trifluoroethane (HCFC-123) is manufactured commercially for use as a refrigerant in large-scale air conditioning units, a foam blowing agent, and a cleaning solvent. HCFC-123 may have several new uses as a replacement for chemicals that are being phased out under the Montreal Protocol due to suspected ozone depletion. It is considered a prime alternative to CFC-11 as a refrigerant in industrial chillers and may be used as a replacement for Halon 1211, a firefighting agent.

HCFC-123 is metabolized through both an oxidative and a reductive pathway (Harris et al., 1991; Brashear et al., 1992). Oxidative metabolism, the major pathway, goes through a trifluoroacetylchloride intermediate that can react covalently to form trifluoroacylated liver proteins or may be hydrolyzed to trifluoroacetic acid (TFA). The minor reductive pathway produces two volatile metabolites: 2-chloro-1,1,1-trifluoroethane (HCFC-133a) and 2-chloro-1,1-difluoroethylene (CDFE). These metabolic pathways are similar to those identified for the anesthetic, halothane, which differs from HCFC-123 only in a bromine for chlorine substitution (Sipes et al., 1980).

An understanding of the relationship between HCFC-123 exposure dose and metabolite formation will be necessary to extrapolate laboratory animal toxicity studies to humans. The objective of this study was to develop a biologically based pharmacokinetic model that describes the tissue and plasma pharmacokinetics of the parent compound, HCFC-123, and the primary oxidative metabolite, TFA, in the Fischer 344 (F-344) rat. A model that adequately describes the tissue dosimetry for HCFC-123 in the rat is the first step toward developing models that can be used to predict the toxicokinetics of HCFC-123 in humans throughout various potential use scenarios.

MATERIALS AND METHODS

Rats

Male F-344 rats (*Rattus norvegicus*) (191 to 354 g) were obtained from Charles River Breeding Laboratories (Kingston, NY). Rats were provided with Purina Formulab #5008 and softened water *ad libitum*. They were housed (two to three per cage) in plastic cages with hardwood chip bedding prior to exposure and were maintained on a 12-h light/12-h dark cycle at constant temperature (22 $\pm$ 1 °C) and humidity (40 to 60%). Cages were changed twice per week and rats were marked for identification with a tail tatoo. All animals were euthanatized by carbon dioxide (CO_2) asphyxiation after the last sample was taken.

Inhalation Exposures and Sample Collection

Rats were exposed to HCFC-123 via inhalation in different exposure systems, depending on whether blood or exhaled breath samples were needed during the exposure. Most rats were exposed to HCFC-123 vapor in a chamber that allowed blood sampling during the inhalation exposure. This chamber is a modification of a chamber designed to collect blood during whole-body dermal exposures to vapors in the presence of respiratory protection (McDougal et al., 1985). Polyethylene jugular cannulae for blood collection were implanted 1 day before the exposure using ketamine/xylazine anesthetic (70 mg ketamine/6 mg xylazine per kg body weight) by the method of Bakar and Niazi (1983). Exposures were performed in a 19.5-L Plexiglas box (internal dimensions — 59 cm × 11 cm × 30 cm) having an air exchange rate of about 20 volumes per hour. Flat expanded steel mesh divided the chamber into six 9.5 cm × 30 cm compartments. The rats were acclimated to the chamber before the exposure and a Velcro harness was used to limit mobility during the exposure, so that jugular canulae could be exteriorized through sealed ports at the top of the chamber. The chamber input air, 6 L/min, was divided and entered the chamber by two baffled ports located in the top. The chamber was exhausted using four ports, two at each end, one near the top and one near the bottom. Groups of four rats were exposed for 4 h to 1.0, 0.1, or 0.01% HCFC-123 by controlling the airflow through liquid HCFC-123 (98.9% pure, Lot #239-1M, Allied-Signal Inc., Morristown, NJ) contained in a gas washing bottle (#7164 250-mL capacity; Ace Glass, Vineland, NJ). The 1% exposure was repeated with a second group of four rats. An ice water bath on the wash bottle was used to maintain a stable temperature of about 0 °C. The generator output was added to the 6 L/min air supply at an angle to the flow to assist mixing. The baffles in the exposure chamber also provided a turbulent mixing in the chamber. Chamber concentrations were monitored with a Miran 1A infrared analyzer (Foxboro Analytical, South Norwolk, CT). The absorption band at 8.6 μm was used for the HCFC-123 analysis, varying the path length and range for different exposure concentrations. The short-path cell (10 cm) was used for the 1% and 0.1% exposures with the range set at 1.0 A and 0.1 A, respectively. A long-path cell set at 2.25 m was used for analyzing the 0.01% exposure using the 0.25 A range. After exposure, rats were placed in metabolism cages for urine collection. Blood samples were drawn periodically from jugular cannulae during and after exposure.

Fifteen rats were exposed to HCFC-123 vapor in a 22.5-L glass bell jar to determine postexposure blood and fat concentrations with a serial sacrifice over 8 h. These rats were exposed for 4 h to 0.1% HCFC-123 using flows of about 8 to 10 L/min. Rats were sacrificed immediately and up to 8 h postexposure. The abdomen was opened, and whole blood and fat samples were harvested. We collected blood from the posterior vena cava using a polyethylene syringe and 23 gauge needle. Triplicate 100-μL aliquots of blood were placed in preweighed 20-mL glass vials. The vials were capped with Teflon/rubber septa and aluminum caps,

reweighed, and placed in a freezer at -40 °C until analyzed. Fat tissue was harvested next by clipping triplicate 100-mg samples from the perirenal area and was handled in a similar manner to the blood samples. The time from sacrifice of the animal to capping of the last vial was maintained between 30 and 40 s to reduce loss of chemical during exposure to air.

Six urethane-anesthetized rats were exposed to HCFC-123 vapor via tracheal cannulae to allow quantitation of delivered dose and expired air. Urethane (Aldrich Chemical Co., Milwaukee, WI) was made up as a 0.2 g/mL solution and given intraperitoneally at 1.25 g/kg. In these experiments, rats were ventilated with 1.0, 0.1, or 0.01% HCFC-123 through a tracheal cannula using a ventilator pump Model 680 (Harvard Apparatus, Millis, MA) for controlling the air exchange rate of anesthetized rats at 140 to 150 mL/min. Two rats were exposed at each concentration for 2 h. The pump was connected to a Tedlar bag containing 80 L of the desired concentration (1.0, 0.1, or 0.01% HCFC-123 in air V/V). Rats were tracheotomized with a 3.5-mm outside diameter, thin-walled, stainless steel cannula, which was inserted to just above the tracheal bifurcation and tied in place. The other end of the tracheal cannula was connected to the "Y" fitting connected to the ventilator tubing lines. The exhaust side of the pump vented to a second "waste" bag and to a loop-injected gas chromatograph (GC) (Varian 3400, Varian Associates Inc., Sunnyvale, CA) for analysis of the exhaled air. Breath analyses were performed using a Varian 3400 GC. The GC was set up with a 0.25-mL loop-injection system, an SPB-5 bonded phase 15 m × 0.53 mm fused silica column, and a flame ionization detector (FID). The analysis was performed isothermally at 150 °C.

Analysis of Biological Samples

Blood and fat samples were collected and capped in 20-mL headspace vials. Vials with fat samples were injected with 0.5 mL of 0.4 g NaOH/mL water and were vortex mixed at 50 °C for 3 to 4 h to digest the tissues. All sample vials then were heated to drive HCFC-123 into the headspace. Samples were diluted as necessary to avoid overloading the detector. Headspace analysis of HCFC-123 in blood and fat was performed on a Varian 3700 GC equipped with an electron capture detector (Varian Associates, Walnut Creek, CA) and a Tekmar Model 7050 automated headspace sampler fitted with a cryofocusing module. Separation was performed on a PoraPLOT Q capillary column (30 m × 0.5 mm) supplied by Chrompack, Inc. (Raritan, NJ).

Trifluoroacetic acid was derivatized to its methyl ester using dimethyl sulfate (Maiorino et al., 1980) and was analyzed in blood and urine using an HP19395A headspace sampler (Hewlett-Packard, Avondale, PA), which was interfaced to a Tekmar Capillary Interface Model 1000 cryofocusing module (Tekmar, Cincinnati, OH). The cryofocusing module was connected to a Hewlett-Packard 5890 GC interfaced to a

Hewlett-Packard 5970 mass selective detector. Separation was performed on a PoraPLOT Q capillary column (30 m × 0.32 mm) supplied by Chrompack, Inc. (Raritan, NJ). A Haake-Buchler vortex evaporator (Saddlebrook, NJ) was used for heating and vortexing hydrolysis samples and derivatization mixtures.

Partition Coefficients

Partition coefficients were determined with a modified version of the vial-equilibration technique described by Gargas et al. (1989). Whole tissue was harvested and minced into a tissue slurry, rather than being prepared as a tissue homogenate in saline. Rats for partition coefficient determinations were sacrificed with CO_2, and whole blood was collected from the posterior vena cava using a heparinized syringe. Liver (L), quadriceps muscle (M), epididymal and perirenal fat (F), and stomach and small intestine from the gastrointestinal tract (G) also were removed for analysis. Blood samples (1.0 mL) were placed in 12.4-mL glass vials and incubated/mixed for 3 h at 37 °C with 800 ppm of chemical in the vial headspace. Whole tissue samples (L and M: 1.0 g; F and G: 0.5 g) were minced and incubated/mixed under the same conditions as for blood, except fat was equilibrated for 8 to 12 h. There were no differences between epididymal and perirenal fat or stomach and small intestine, so the partition coefficients were combined. Chemical concentrations in headspace were analyzed with an HP19395A headspace sampler, connected to an HP5890 GC (Hewlett-Packard, Palo Alto, CA), which was equipped with a hydrogen FID and 6 ft ×1/8 in. stainless steel OV-101 WHP 100/120 column. The GC conditions were set at a detector temperature of 250 °C, injection temperature of 125 °C, and nitrogen carrier gas flow of 30 mL/min. The oven temperature was held constant at 85 °C for blood, liver, muscle, and fat samples and at 50 °C for gastrointestinal samples.

Metabolic Constants

A closed-chamber gas uptake system with a volume of 8.5 L was used for determination of metabolic constants. Three rats (per exposure) were exposed to the study chemical using a closed recirculating gas uptake system similar to that described by Gargas et al. (1986). Seven exposures were performed for 6 h each at starting concentrations of 0.012, 0.062, 0.107, 0.215, 0.31, 0.56, and 1.11%. Ascarite (150 g) was used as the CO_2 absorber. Oxygen concentrations were maintained at 21% (± 1%) during the exposures. Flow to the exposure system was maintained at 2.1 L/min and to the sample loop of the GC at 120 mL/min.

The chemical concentrations in the chamber atmosphere were monitored every 5 min for the first 30 min and every 15 min thereafter, using a gas sampling valve connected to an HP5890 GC. Chromatography was performed on a 12 ft×1/8 in. stainless steel column packed with 10% SE30 on 80/100 mesh Chromosorb W-HP (Supelco, Bellefonte, PA). The GC was equipped with a hydrogen FID with temperature set at 250 °C,

nitrogen carrier flow at 30 mL/min, injection temperature at 125 °C, and oven temperature held constant at 85 °C.

Mathematical Model

A PBPK model (Figure 3.2-1) was developed with the general form of Ramsey and Andersen (1984) plus a fat compartment that becomes a greater proportion of body weight with increasing weight; a gut compartment that has flow into the liver compartment; a single nonphysiologic compartment to describe the volume of distribution of the oxidative metabolite, TFA (Fisher et al., 1989); and an enzymatic term that incorporates a decrease, in addition to saturation, of metabolism at high concentrations. The differential equations for the PBPK model are given in the appendix. Simusolv (Mitchell & Gauthier Associates, Concord, MA), a FORTRAN-based continuous simulation language with optimization capabilities, was used on a VAX 8530 (Digital Equipment Corp., Maynard, MA) for all simulations.

Model Parameters

Physiological and biochemical parameters used for the model are shown in Table 3.2-1. Compartment volumes, except for fat, are derived from Delp et al. (1991). Anderson et al. (1993) showed that the fat compartment of F-344 rats becomes a larger percentage of body weight with aging. Anderson's equation was used to determine the size of the fat compartment for a 100-g body weight. The percentages for the other compartments were calculated based on the fat-free body weight. Cardiac output and alveolar ventilation rates (Arms and Travis, 1988) were scaled to body weight to the exponent 0.75. Blood/air and tissue/air partition coefficients were experimentally obtained as described above. Metabolic constants were fit from the closed chamber gas uptake data (Figure 3.2-2) and scaled to body weight using the allometric relationship described by Andersen et al. (1987a).

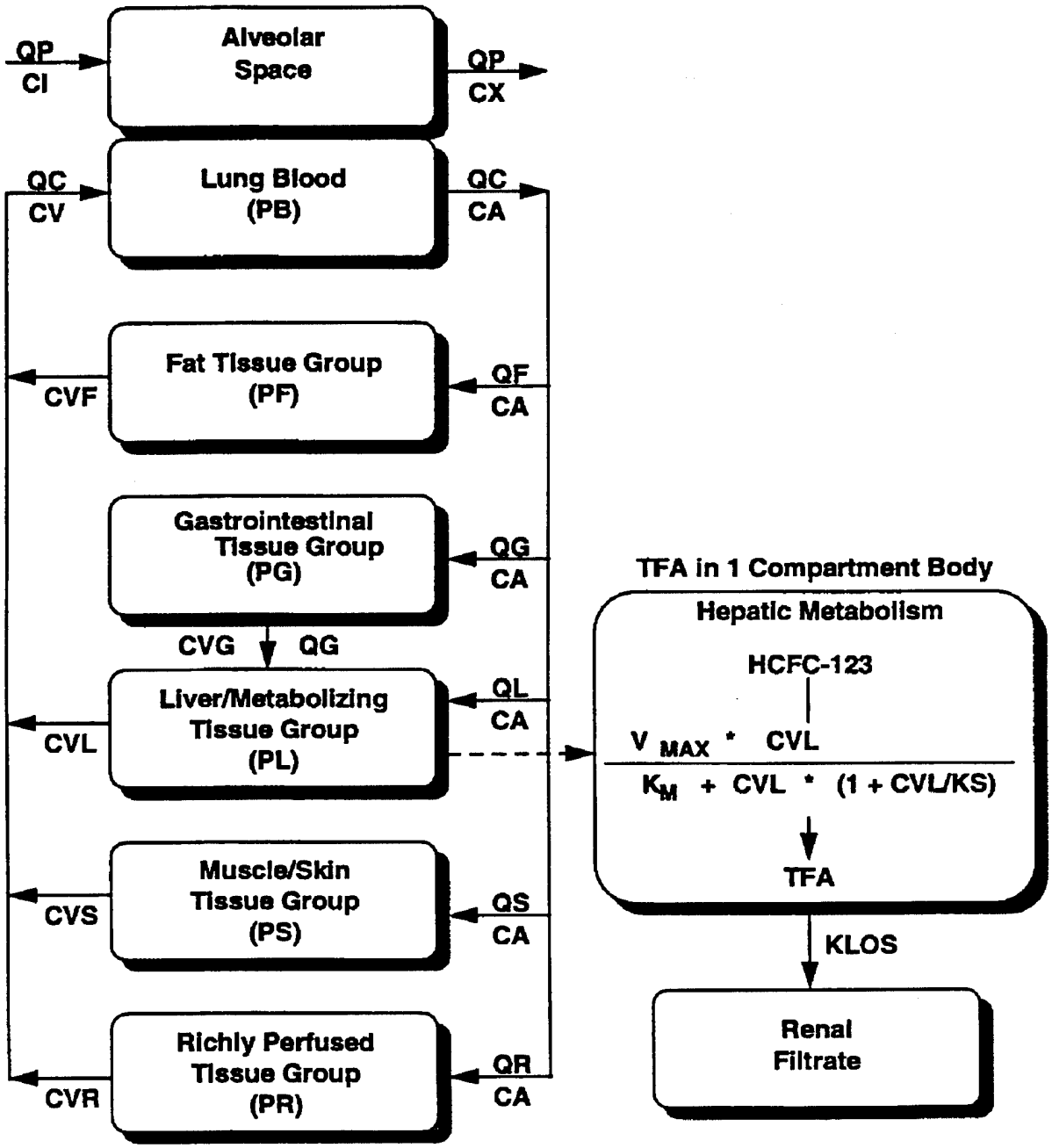


Figure 3.2-1. Physiologically Based Pharmacokinetic Model for HCFC-123 with a One-Compartment Classical Model for Its Metabolite TFA. Abbreviations: CA, arterial concentration; CX, exhaled concentration; CI, inhaled concentration; CV, venous concentration; CVF, venous fat concentration; CVG, venous gastrointestinal concentration; CVL, venous liver concentration; CVS, venus slowly perfused concentration; CVR, venous richly perfused concentration; QP, alveolar ventilation; QC, cardiac output; QF, fat blood flow; QG, gastrointestinal blood flow; QL, liver blood flow; QS, slowly perfused blood flow; QR, richly perfused blood flow; V_{MAX}, maximum rate of metabolism; K_M, Michaelis-Menten constant; KS, metabolic suppression constant; KLOS, first-order excretion rate of TFA.

TABLE 3.2-1. PARAMETERS USED IN THE PHYSIOLOGICALLY BASED MODEL FOR HCFC-123

Parameter	Value
Body Weight Range (kg)	0.191 - 0.354
Volumes	
Slow (% of Fat Free Body Weight)	70.6
Rapid (% of Fat Free Body Weight)	5.3
Liver (% of Fat Free Body Weight)	4.0
Gut (% of Fat Free Body Weight)	3.6
Fat Range (% of Body Weight)[1]	8.8 - 14.5
Flows	
Alveolar Ventilation Rate (L/h/kg)[2]	19.9
Cardiac Output (L/h/kg)	14.1
Slow (% of Cardiac Output)	35.1
Rapid (% of Cardiac Output)	43.4
Liver (% of Cardiac Output)	1.5
Fat (% of Cardiac Output)	6.1
Gut (% of Cardiac Output)	13.9
Partition Coefficients[3]	
Slowly Perfused/Air	2.1 ± 0.3
Rapidly Perfused/Air	3.3 ± 0.3
Liver/Air	3.3 ± 0.3
Gut/Air	3.1 ± 1.1
Fat/Air	70.3 ± 3.7
Blood/Air	3.2 ± 0.4
Metabolic Constants	
Maximum Metabolic Rate - V_{max} (mg/h/kg)	8.8
Michaelis-Menten Constant - K_m (mg/L)	0.7
Suppression Constant - K_s (mg/L)	65.0
TFA Parameters	
Volume of Distribution (L)	0.345
Renal Elimination Rate ($h^{-1}kg^{-1}$)	0.01

[1] The volume of the fat compartment constant was calculated by 0.01 (35 × body weight + 2.1), and the other compartments were constant proportions of the fat free body weight (see text).

[2] 14 L/h was used for gas uptake simulations, and 20.9 L/h was the measured mean in the studies where rats were artificially ventilated.

[3] Values are mean $\pm$ SD.

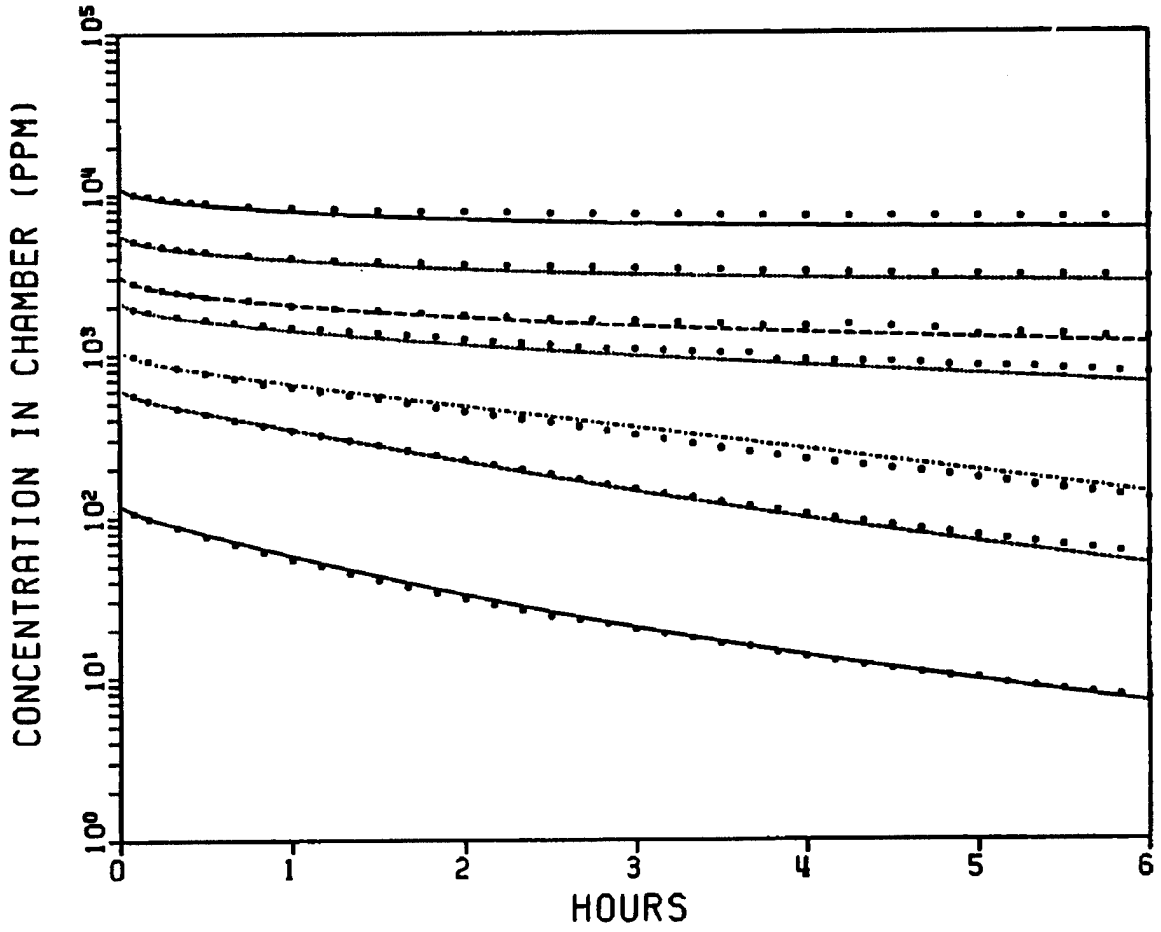


Figure 3.2-2. Gas Uptake Data for HCFC-123 Collected for 6 h with Starting Concentrations of 1.11, 0.56, 0.31, 0.215, 0.107, 0.062, and 0.012%. (Symbols represent actual measurements; continuous lines represent the simulations.)

Model Development

A flow-limited model structure for volatile chemicals based on Ramsey and Andersen (1984) was used as the starting place for a model of HCFC-123. A gut compartment, making up about 3% of body weight and receiving about 14% of the total cardiac output was added to provide a more physiological representation of the splanchnic compartment in the rat (Figure 3.2-1). Lumped compartment model parameters were based on a recent publication (Delp et al., 1991), which measured blood flows and organ masses in Sprague-Dawley rats. The mass of the slow compartment, in the model, was comprised of muscle and skin and the mass of the rapid compartment was comprised of heart, brain, kidney, and reproductive tissue. Contributions from the "other" category were lumped into the mass of the slow compartment. Although this category was not completely defined, flows to bone and "other" were distributed between slow and rapid compartment flows

because it appeared that some of the tissues lumped into the "other" category were more appropriate in the rapid compartment. This gave the rapid and slow flows values that were more consistent with previous models.

The portion of the model that describes TFA distribution and elimination is based on classical pharmacokinetic analysis. The disposition of TFA in the model is controlled by the rate of HCFC-123 metabolism to TFA (V_{max}, K_m, and K_s), the apparent volume of distribution for TFA in the body, and the rate of renal elimination for TFA. The model assumes that TFA formation and elimination occur solely by metabolism in the liver and excretion from the kidney, respectively. The elimination rate constant was allometrically scaled to body weight to the exponent -0.25.

The following procedure was used to estimate the apparent volume of distribution for TFA. The first order loss rate of TFA was set equal to the negative value of the slope of the linear terminal phase of the plasma concentration versus time data obtained from the three sets of TFA data resulting from the inhalation exposures. For purposes of determining the volume of distribution, it was assumed that all of the metabolism of HCFC-123 was oxidative and resulted in the formation of TFA. The rate of formation for TFA was based on the metabolic constants describing the metabolism of HCFC-123 that were obtained from the gas uptake data for HCFC-123. It is recognized that some small fraction of the oxidative biotransformation of HCFC-123 results in the formation of trifluoroacylated liver proteins and that some small fraction of HCFC-123 is transformed reductively to the volatile metabolites HCFC-133a and CDFE (Sipes et al., 1980; Brashear et al., 1992).

An observed decrease in TFA levels in venous blood during and after exposure to HCFC-123 at 1% was described by incorporating suppression of the metabolism (K_S) of HCFC-123 at high concentrations. Gas uptake data alone were not sensitive enough to detect the reduced rate of disappearance of HCFC-123 at 1%; however, based on the TFA blood concentrations, the suppression constant was estimated. Figure 3.2-3 shows the effect of the suppression constant on the TFA venous blood simulation. Although the mechanism of substrate inhibition has not been determined, the description used in the model is taken from the generalized scheme and equation for multiple mechanisms of substrate interactions described by Andersen et al. (1987b). Initially, V_{max} and K_m were determined without suppression. The model then was used to simulate the TFA data and derive the suppression constant. Final values of V_{max} and K_m then were determined by using the gas uptake data with the estimated suppression constant.

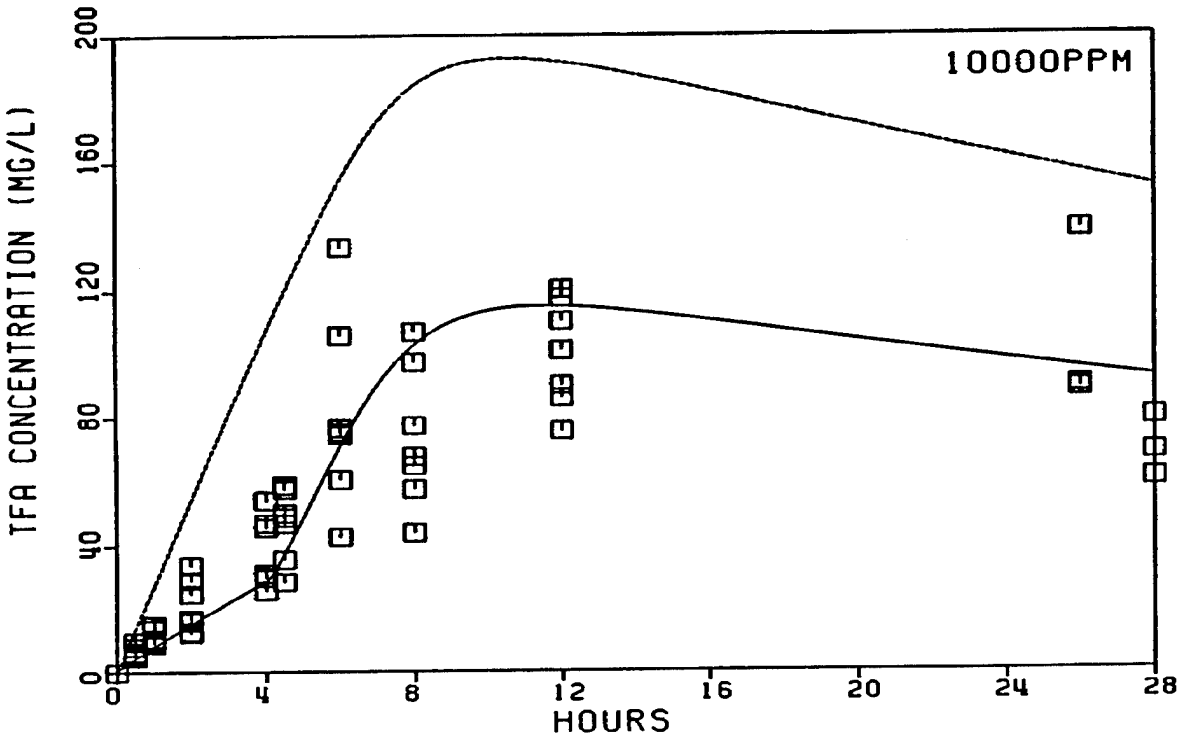


Figure 3.2-3. Concentrations of TFA in Venous Blood during and after a 4-h Exposure to 1.0% HCFC-123. (Symbols represent actual measurements for the same individual animals at each time; the upper and lower continuous lines represent, respectively, the simulation without and with a term describing the suppression of HCFC-123 metabolism to TFA.)

RESULTS

Partition Coefficients

The rat tissue/air partition coefficients determined for HCFC-123 that were used in the PBPK model optimization to derive metabolic constants are shown in Table 3.2-1. Time-to-equilibration studies and vial headspace concentration studies were performed. No evidence of dose-dependent partitioning was seen. Time-to-equilibration studies showed an increase in the uptake of chemical for fat tissue from 1 to 8 h, and then a plateau in the partition value from 8 to 12 h. All other tissues were equilibrated for 3 h with no change in the partition value over time.

Gas Uptake Studies

The inhalation uptake of HCFC-123 by the rat showed two discernable phases: a rapid equilibration phase that lasted 30 to 45 min followed by a slow linear uptake phase for the duration of the exposure

(Figure 3.2-2). Using a simulation approach to optimize for metabolic constants, with fixed physiological and solubility parameters, the set of gas uptake curves was adequately described by a single saturable pathway.

Pharmacokinetics of HCFC-123

Blood concentrations of HCFC-123 during and after exposure to 1.0, 0.1, or 0.01% HCFC-123 for 4 h (Figure 3.2-4) rose rapidly and apparently reached steady state by the end of the 4-h exposure and then fell rapidly at the end of the exposure. Blood concentrations of the oxidative metabolite TFA rose during the exposure and continued to rise postexposure, with very little decrease seen even 24 h after the exposure. Concentrations of TFA during the 1.0% exposure were lower than those achieved from the 0.1% exposure. Urinary TFA concentrations appeared to almost linearly increase up to 48 h postexposure (Figure 3.2-5).

Postexposure Blood and Fat Concentrations

Blood (sampled from the femoral vein) and fat concentrations from rats serially sacrificed for 8 h after a 4-h exposure to 0.1% HCFC-123 decreased rapidly (Figure 3.2-6). Venous concentrations dropped more steeply immediately after exposure and then appeared to be dependent on the kinetics of the fat compartment.

Expired Breath Concentrations

Expired breath concentrations of HCFC-123 from anesthetized, ventilated animals exposed for 2 h to 1.0, 0.1, or 0.01% plateaued within 10 min of the onset of exposure (Figure 3.2-7). Postexposure concentrations showed an initial rapid decrease, but did not start flattening out until at least 1 h postexposure. Problems with ventilating the animals during the 1.0% exposures resulted in incomplete data collection.

Simulation of Data

The model did a good job of simulating blood data spanning over three orders of magnitude, from exposures to HCFC-123 that spanned two orders of magnitude (Figure 3.2-4). The simulation slightly overestimates the measured blood concentrations during the exposure at all three concentrations. The immediate drop postexposure is well simulated, but the simulation underestimates the measured blood concentrations late in the postexposure period, especially at the lowest concentration.

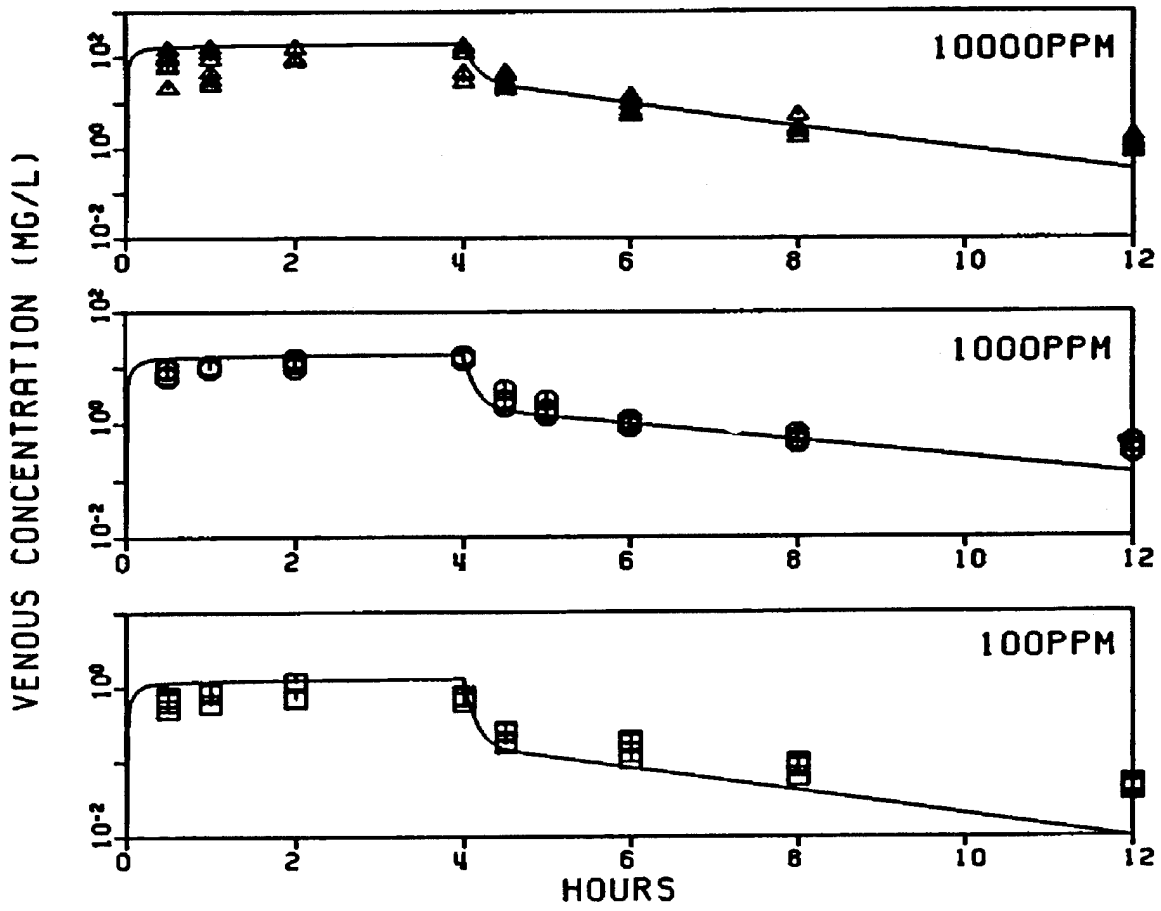


Figure 3.2-4. Concentrations of HCFC-123 in Venous Blood during and after 4-h Exposures to 1.0, 0.1, and 0.01%. (Symbols represent actual measurements for the same individual animals at each time; continuous lines represent the simulations.)

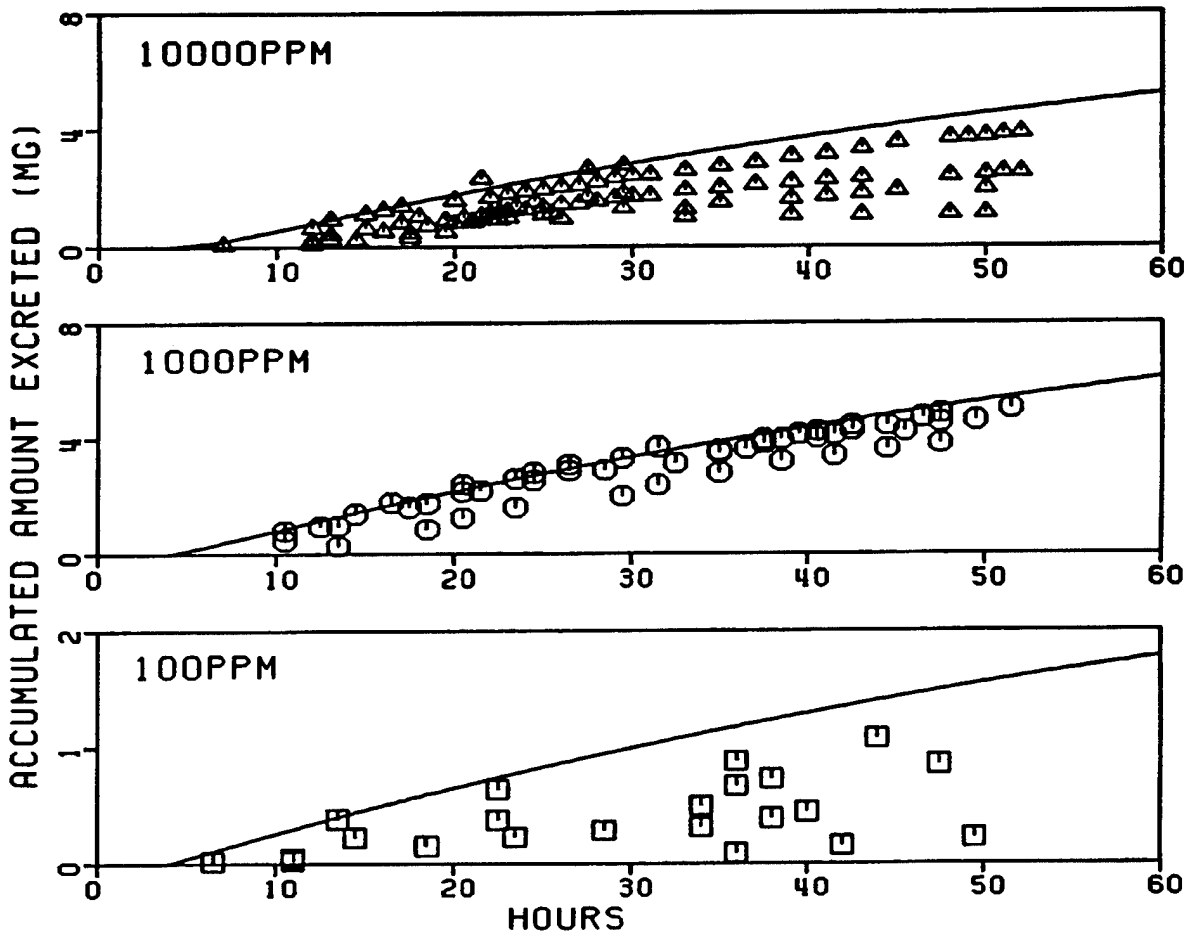


Figure 3.2-5. Accumulative Amount of TFA in Urine after 4-h Exposures to 1.0, 0.1, and 0.01% HCFC-123. (Symbols represent actual measurements for the same individual animals at each time; continuous lines represent the simulations.)

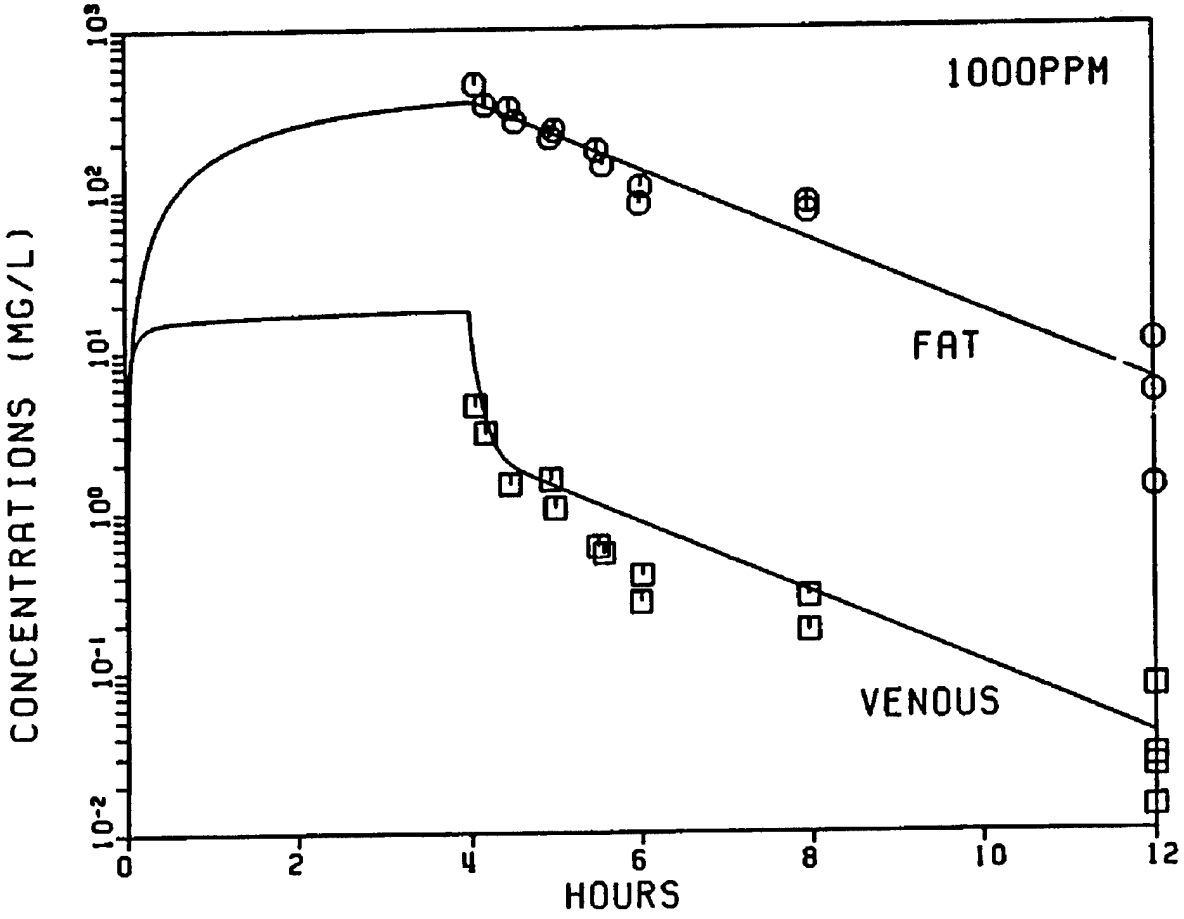


Figure 3.2-6. Concentrations of HCFC-123 in Fat and Venous Blood during and after a 4-h Exposure to 0.1%. (Symbols represent actual measurements for different individual animals at each time; continuous lines represent the simulations.)

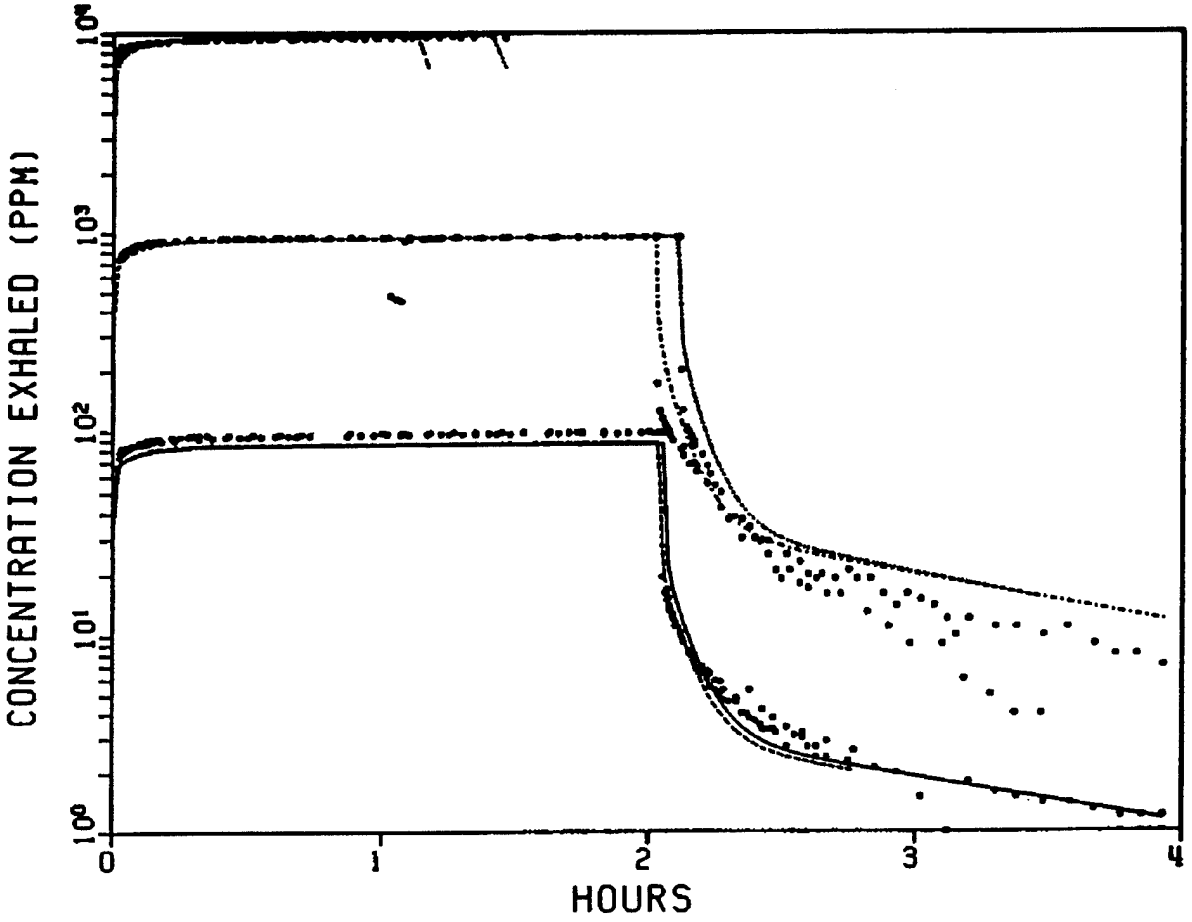


Figure 3.2-7. Concentrations of HCFC-123 in Exhaled Breath during and after 2-h Exposures to 1.0, 0.1, and 0.01%. (Symbols represent actual measurements; continuous lines represent the simulations.)

Fat and venous blood concentrations resulting from a bell jar exposure also were well estimated by the model (Figure 3.2-6). Both sets of data are simulated fairly accurately during the 8-h postexposure period.

Trifluoroacetic acid concentrations in blood after 4-h exposures were well simulated when the appropriate term for describing the suppression of HCFC-123 metabolism was included in the model (Figure 3.2-8). Simulations of TFA in venous blood continue to be accurate to 100 to 150 h.

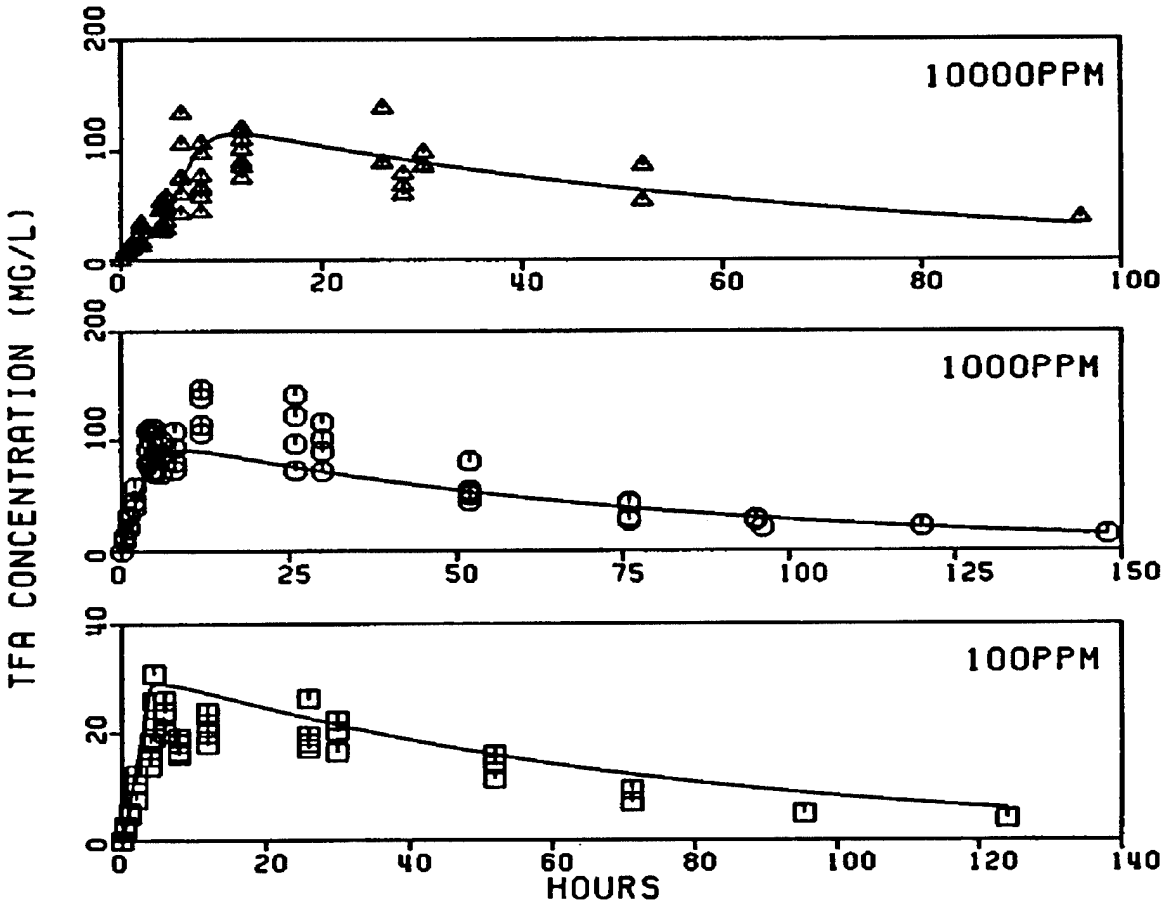


Figure 3.2-8. Concentrations of TFA in Venous Blood during and after 4-h Exposures to 1.0, 0.1, and 0.01% HCFC-123. (Symbols represent actual measurements for the same individual animals at each time; continuous lines represent the simulations.)

Cumulative TFA concentrations in urine postexposure were simulated in Figure 3.2-5. At each exposure, the simulation was higher than the experimental measurements. The 0.1% concentration was most accurately simulated, but the high and low exposures were overestimated by the model.

Expired concentrations of HCFC-123 also were reasonably simulated by the model (Figure 3.2-7). At the low concentration (0.01%), the exhaled breath measurements are slightly higher than the simulation, but the 2-h postexposure phase is fairly close. At the intermediate concentration (0.1%), the exhaled breath concentrations during the exposure are matched, but the slope of the postexposure phase of the measured data is slightly different than the simulation. Overall, the model does an adequate job of simulating both HCFC-123 and its oxidative metabolite TFA in the male F-344 rat.

DISCUSSION

Pharmacokinetic studies of HCFC-123 in laboratory animals are limited. There are no published studies of blood concentrations of HCFC-123 after exposures. In the only relevant published study of TFA formed after HCFC-123 exposures to rats for 6 h to 1.0, 0.1, or 0.01% HCFC-123 excreted 82.9 $\pm$ 18.9, 87.2 $\pm$ 21.0, and 13.8 $\pm$ 2.4 μmol of TFA per kg rat, respectively, in the 12 h following the end of the exposures (Harris et al., 1992). In the current study, rats exposed for 4 h to 1.0, 0.1, or 0.01% HCFC-123 excreted 31.9 $\pm$ 17.1, 42.3 $\pm$ 5.1, and 7.9 $\pm$ 3.2 μmol of TFA/kg rat, respectively, in the 12 h following the end of the exposures. The lower urinary yield of TFA in the current study is consistent with the shorter exposure period to HCFC-123.

An initially surprising outcome of the pharmacokinetic study of HCFC-123 was the observation that the production of TFA was suppressed during exposure to high concentration of the parent compound, with the consequent need to describe this metabolic suppression in the PBPK model. The suppression of oxidative metabolism was not apparent from the simulation of the closed chamber data, but was very apparent from the metabolite blood concentrations. In the simulation of the 1% closed-chamber data, the K_s of 65 mg/L only decreased the loss from the chamber at 4 h by 4.6%, a value that would probably be lost in experimental variation. However, in simulations of blood TFA data for a 4-h 1% constant exposure, the suppression constant decreased the TFA blood concentrations by 73% (from 107.0 to 29.0 mg/L). These simulations explain why suppression is not detectable from closed chamber experiments.

The similarity in structure and the metabolites in common make halothane a useful chemical to compare with HCFC-123 with regard to metabolism. It has been suspected for some time that halothane is capable of suppressing its own metabolism. However, some experimental protocols were not capable of distinguishing enzyme suppression from enzyme saturation, as was the case in an early experiment in which swine were exposed to concentrations of halothane from 0.0006 to 1.6% (Sawyer et al., 1971) and a more recent experiment in which dogs were exposed to concentrations of halothane of 0.45 or 1.21% (Sakai et al., 1991).

Definitive suppression of TFA formation during halothane exposure has been shown in two reported studies. In one study, suppression of the formation of TFA was demonstrated in rats during exposure to 1.5% halothane for 10 min followed by additional exposure to 1.0% for either 50 min or 5 h and 50 min. Trifluoroacetic acid in serum was measured immediately after the 1- and 6-h exposures and 5 h after the 1-h exposure. The serum levels of TFA were 12.3, 38.4, and 77.9 mg/L, respectively. Levels at the end of the 6-h exposure being about half of those measured 5 h after the end of a 1-h exposure support the hypothesis that enzyme inhibition rather than enzyme saturation was the limiting factor for halothane metabolism (Eckes and Büch, 1985).

In a second study, guinea pigs exposed to 1.0 or 0.1% halothane for 4 h had plasma concentrations of TFA immediately after exposure of 418 $\pm$ 99 and 948 $\pm$ 141 μM, respectively. By 10 h postexposure, concentrations of TFA were 1970 $\pm$ 253 and 1268 $\pm$ 150 μM, respectively (Lind and Gandolfi, 1992, 1993). The authors concluded that halothane appears to inhibit its own biotransformation at high concentrations, but that as hepatic concentrations decrease after exposure, there is some rebound of metabolism.

A mechanism to explain the observed enzyme inhibition has not been offered by previous authors. Some insight is offered indirectly by Clark and Tinston (1982), who compared the potency of a series of halogenated and nonhalogenated hydrocarbons in causing central nervous system effects (either stimulation or depression) in rats and cardiac sensitization in dogs. When the concentration producing an effect in 50% of the animals (EC_{50}) for these effects was expressed as volumes percent in air, the potencies of this wide range of chemicals differed greatly. However, when the toxicities were expressed thermodynamically (relative saturation at EC_{50}), the potencies were similar. The authors concluded that these rapidly reversible effects were caused by "physical toxicity" (Ferguson, 1939), in which a chemical's presence in some part of the cell temporarily disorganized its function.

An extension of the concept of physical toxicity suggests several possible ways in which halothane and HCFC-123 might act to inhibit their own metabolism. Both, being lipophilic, would tend to be taken up in the lipid matrix of biological membranes, where they could directly affect the fluidity of the membrane, which could potentially alter the function of membrane-bound proteins (Goldstein, 1984). Specifically, configurational changes in the P_{450} or reduced nicotinamide-adenine dinucleotide phosphate (NADPH)-P_{450} oxidoreductase, or in the electron transfer from one to the other might result from the membrane fluidity change. Also, because of being lipophilic, they might be attracted directly to lipophilic sites of the P_{450} or NADPH-P_{450} oxidoreductase, resulting in configurational changes. Any of the suggested interactions has the potential for affecting the rate of metabolism of the parent compound to TFA. Furthermore, rapidly

decreasing concentrations of parent chemical postexposure would allow reversal of the suppression of enzyme activity and subsequent increase in TFA production.

The PBPK modeling approach used here is the most useful way to put laboratory animal studies into a framework that can be used to understand the underlying biochemical and mechanistic processes that may affect toxicity. The recognition of the suppression of HCFC-123 metabolism at high exposure concentrations and the incorporation of the suppression into the PBPK model made it capable of simulating the production and kinetics of the major metabolite (TFA) as well as the parent compound. Questions can now be asked about whether it is the parent or metabolite that relates to the toxic effects that have been identified. This type of model can assist in designing experiments that will further the quantitative understanding of toxicity. With appropriate human metabolic parameters and partition coefficients, which can be estimated *in vitro*, the model could be used to assess the kinetic disposition of HCFC-123 in humans. The ultimate payoff may be improved safety standards that are based on quantitative extrapolations between species, doses, and routes.

APPENDIX

The mass balance differential equations describing each of the tissue compartments in the model for HCFC-123 are as follows.

For simple well-stirred compartments where neither metabolism nor other losses occurred (richly and slowly perfused tissues, fat, and gut), the equation was

$$\frac{dA_i}{dt} = Q_i (C_A - C_{Vi})$$

$$C_{vi} = \frac{C_i}{P_i}$$

where i represents the ith compartment, A_i represents the amount in the ith compartment, Q_i represents the blood flow through the ith compartment, C_A represents the arterial concentration, C_{Vi} represents the venous concentration leaving the ith compartment, C_i represents the concentration in the ith compartment, and P_i represents the tissue/blood partition for the ith compartment.

The liver was described as above, but with provisions for blood flow from the gut and for metabolism of the compound

$$\frac{dAM}{dt} = \frac{V_{max}\ (C_{VL})}{K_m + C_{VL}\ (1 + \frac{C_{VL}}{K_s})}$$

$$\frac{dA_L}{dt} = Q_L(C_A - C_{VL}) + Q_G(C_{VG} - C_{VL}) - \frac{dAM}{dt}$$

where dAM/dt is the rate of metabolism, V_{max} is the maximum rate of metabolism, K_m is the Michaelis-Menten constant, and K_s is the suppression constant.

The TFA production (dA_{TFA}/dt) is expressed as a percent (*PCTFA*) of the rate of metabolism of HCFC-123. The enzymatic conversion of HCFC-123 to TFA results in a stoichiometric decrease in molecular weight (*MWT*) proportionate to the ratio of molecular weights (*MWT* = 114.02/152.93) of TFA to that of HCFC-123.

$$\frac{dA_{TFA}}{dt} = PCTFA\ (\frac{dAM}{dt})\ MWT$$

The rate of change in the amount of TFA in the blood (dA_{TFAb}/dt) is described by the TFA production term and a first-order elimination term, where V_d is the volume of distribution of TFA, C_{TFAb} is the concentration of TFA in the blood, and *KLOS* is the elimination rate.

$$\frac{dA_{TFAb}}{dt} = \frac{dA_{TFA}}{dt} - V_d(C_{TFAb})\,KLOS$$

REFERENCES

Andersen, M.E., H.J. Clewell III, M.L. Gargas, F.A. Smith, and R.H. Reitz. 1987a. Physiologically based pharmacokinetics and the risk assessment process for methylene chloride. *Toxicol. Appl. Pharmacol.* 87:185-205.

Andersen, M.E., M.L. Gargas, H.J. Clewell III, and K.M. Severyn. 1987b. Quantitative evaluation of the metabolic interactions between trichloroethylene and 1,1-dichloroethylene *in vivo* using gas uptake methods. *Toxicol. Appl. Pharmacol.* 89:149-157.

Anderson, Y.B., J.A. Jackson, and L.S. Birnbaum. 1993. Maturational changes in dermal absorption of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in Fischer 344 rats. *Toxicol. Appl. Pharmacol.* 119:214-220.

Arms, A.D. and C.C. Travis. 1988. Reference physiological parameters in pharmacokinetic modeling. EPA/600/6-88/004. Office of Health and Environmental Assessment, U.S. Environmental Protection Agency, Washington, DC.

Bakar, S.K. and S. Niazi. 1983. Simple reliable method for chronic cannulation of the jugular vein for pharmacokinetic studies in rats. *J. Pharmaceut. Sci.* 72:1027-1029.

Brashear, W.T., M.M. Ketcha, D.L. Pollard, C.S. Godin, H.F. Leahy, P.P. Lu, E.R. Kinkead, and R.E. Wolfe. 1992. Metabolite identification of halon replacement compounds. AL-TR-1992-0078. Wright-Patterson Air Force Base, OH: Armstrong Laboratory.

Delp, M.D., R.O. Manning, J.V. Bruckner, and R.B. Armstrong. 1991. Distribution of cardiac output during diurnal changes of activity in rat. *Am. J. Physiol.* 261:H1487-H1493.

Clark, D.G. and D.J. Tinston. 1982. Acute inhalation toxicity of some halogenated and non-halogenated hydrocarbons. *Human Toxicol.* 1:239-247.

Eckes, L. and H.P. Büch. 1985. Influence of disulfiram, diethyldithiocarbamate and carbon disulfide on the metabolic formation of trifluoroacetic acid from halothane in the rat. *Anzneim.-Forsch./Drug Res.* 35:1447-1451.

Ferguson, J. 1939. The use of chemical potentials as indices of toxicity. *Proc. Royal. Soc. B.* 127:387-404.

Fisher, J.W., T.A. Whittaker, D.H. Taylor, H.J. Clewell III, and M.E. Andersen. 1989. Physiologically based pharmacokinetic modeling of the pregnant rat: A multiroute exposure model for trichloroethylene and its metabolite, trichloroacetic acid. *Toxicol. Appl. Pharmacol.* 99:395-414.

Gargas, M.L., M.E. Andersen, and H.J. Clewell III. 1986. A physiologically based simulation approach for determining metabolic constants from gas uptake data. *Toxicol. Appl. Pharmacol.* 86:341-352.

Gargas, M.L., R.J. Burgess, D.E. Voisard, G.H. Cason, and M.E. Andersen. 1989. Partition coefficients of low-molecular-weight volatile chemicals in various liquids and tissues. *Toxicol. Appl. Pharmacol.* 98:87-99.

Goldstein, D.B. 1984. The effects of drugs on membrane fluidity. *Ann. Rev. Pharmacol. Toxicol.* 24:43-64.

Harris, J.W., L.R. Pohl, J.L. Martin, and M.W. Anders. 1991. Tissue acylation by the chlorofluorocarbon substitute 2,2-dichloro-1,1,1-trifluoroethane. *Proc. Natl. Acad. Sci. USA* 88:1407-1410.

Harris, J.W., J.P. Jones, J.L. Martin, A.C. LaRosa, M.J. Olson, L.R. Pohl, and M.W. Anders. 1992. Pentahaloethane-based chlorofluorocarbon substitutes and halothane: Correlation of *in vivo* hepatic protein trifluoroacetylation and urinary trifluoroacetic acid excretion with calculated enthalpies of activation. *Chem. Res. Toxicol.* 5:720-725.

Lind, R.C. and A.J. Gandolfi. 1992. Concentration dependent inhibition of halothane biotransformation and covalent binding in the guinea pig. *The Toxicologist* 12:63.

Lind, R.C. and A.J. Gandolfi. 1993. Concentration-dependent inhibition of halothane biotransformation in the guinea pig. *Drug Metabol. Disp.* 21:386-389.

Maiorino, R.M., A.J. Gandolfi, and I.G. Sipes. 1980. Gas chromatographic method for the halothane metabolites, trifluoroacetic acid and bromide, in biological fluids. *Analytic. Toxicol.* 4:250-254.

McDougal, J.N., G.W. Jepson, H.J. Clewell III, and M.E. Andersen. 1985. Dermal absorption of dihalomethane vapors. *Toxicol. Appl. Pharmacol.* 79:150-158.

Ramsey, J.C. and M.E. Andersen. 1984. A physiologically based description of the inhalation pharmacokinetics of styrene in rats and humans. *Toxicol. Appl. Pharmacol.* 73:159-175.

Sakai, T., H. Yoshida, A. Fukui, and M. Takaori. 1991. Urinary and biliary excretion of metabolites of halothane in dogs. *Drug Metabol. Disposit.* 19:419-422.

Sawyer, D.C., E.I. Eger II, S.H. Bahlman, B.F. Cullen, and D. Impelman. 1971. Concentration dependence of hepatic halothane metabolism. *Anesthesiology* 34:230-235.

Sipes, I.G., A.J. Gandolfi, L.R. Pohl, G. Krishna, and B.R. Brown, Jr. 1980. Comparison of the biotransformation and hepatotoxicity of halothane and deuterated halothane. *J. Pharmacol. Exp. Ther.* 214:716-720.

3.3 DETERMINATION OF BLOOD CONCENTRATIONS FOLLOWING DERMAL EXPOSURE OF CHEMICAL VAPOR

E.R. Kinkead, R.E. Wolfe, S.A. Salins, and D.L. Pollard

ABSTRACT

Selected chemicals used by the Air Force have the potential for systemic toxicity via skin absorption due to exposure to the vapor or gas. Predictions of the chemicals that may be of concern are based on estimates of vapor permeability constants. The objective of this study was to determine blood concentrations in rats during chemical vapor exposure of the skin only. The data will be used in a physiologically based pharmacokinetic model to determine the dermal flux and permeability constants for each of the test substances. Male Fischer 344 rats were placed in whole-body chambers while wearing air-only face masks for vapor exposure of the test substance. During exposure, blood samples were drawn and analyzed for test substance concentration.

INTRODUCTION

During vapor or gas exposures, human absorption of chemicals occurs primarily in the respiratory tract due to the anatomical and functional nature of this region. In most vapor inhalation studies, the significance of dermal uptake is not addressed. However, the skin represents a significant potential route of exposure and could be, under certain occupational conditions, a primary route of exposure. Although it is conventional practice for individuals to take steps to protect their respiratory system when entering an environment of toxic vapors or gases, donning of full body protective gear is not. In these situations, the concern for dermal absorption is increased, and the need for an accurate hazard assessment is warranted.

Efforts to measure whole-body absorption of chemical vapors or gases have been restricted primarily due to the lack of well-defined experimental methods. Recently, a dermal-vapor exposure system employing small rodents as a laboratory animal model was developed by McDougal et al. (1985) to quantify the absorption of chemicals through the skin. The data obtained from this experimental method were used in a physiologically based pharmacokinetic (PBPK) model to determine the dermal flux and permeability constants for a series of volatile organic chemicals (McDougal et al., 1986, 1990). The results from these investigations indicated that this approach provided a conservative estimate of vapor permeability constants in humans.

A need exists to extend the information gained from the dermal-vapor exposure system and PBPK modeling approach developed by McDougal and associates to additional chemicals of toxicologic concern and to further verify the model in its application for human hazard assessment. The Air Force inventory of

chemicals in use includes volatile organics that have a wide range of toxicologic effects. Information regarding the skin absorption of the vapors of these chemicals is needed to determine if the potential for systemic toxicity could result following this route of exposure.

This type of study is relevant to the Air Force because it is designed to determine the rate of vapor penetration for selected Air Force chemicals (which, based on physical and chemical characteristics, might penetrate the skin at a high rate). Chemicals included in this study are relevant for a number of reasons. Vinyl chloride has an Air Force regulation, AFOSH 161-5, concerning its inhalation hazard, but the dermal-only route is not addressed. Carbon tetrachloride is used as a cleaning solvent. Ethyl acrylate is used in acrylic resins and adhesives on aircraft, and trichloroethylene is used as a degreaser. Methyl chloroform is used as a solvent, whereas methylene chloride is used to strip paint. Ethylene dichloride is used as an antiknock fuel additive and engine cleaner. Allyl chloride data are needed to correlate with skin:air partition coefficient data in order to develop an *in vitro* screening method. Isopropanol is used as a wipe solvent in place of ozone-depleting chemicals. *m*-Methyl styrene and *p*-methyl styrene are necessary to complete the database on styrene and for examining the structure–activity relationship of compounds and their skin permeability. Chemicals included in this study have been either in or are currently in the Air Force inventory.

MATERIALS AND METHODS

A detailed description of the exposure methods and experimental evaluations performed for this study were provided in the 1991 Toxic Hazards Research Unit Annual Report (Kinkead et al., 1992).

Blood samples taken from rats during exposure via polyethylene catheters were analyzed using a heptane extraction method. Fresh standard solutions were prepared one or two days prior to each exposure and were stored at -60 °C prior to use. High purity, gas chromatographic-grade heptane that contained no impurities that would interfere with quantification was used. Calibration data were obtained by spiking control blood with standard solutions of the test compound. A Varian (Varian Associates, Palo Alto, CA) model 3700 gas chromatograph (GC) with a GC column (poroPLOT Q, 25-m × 0.53-mm)was used for analysis. This column strongly retains volatile chemicals and provides sufficient separation to eliminate any interference.

RESULTS

Dermal exposures to methylene chloride vapor were conducted using rats cannulated with polyethylene tubing. The exposures were performed at methylene chloride concentrations of 30,000, 60,000, and 100,000 ppm. Blood samples were removed from the rats at 0.5, 1.0, 2.0, and 3.0 h of exposure and immediately prior to the conclusion of the exposure at 4.0 h. Measured methylene chloride blood

concentrations increased with an increase in exposure concentration (Table 3.3-1). Blood concentrations rose rapidly and appeared to reach a near steady-state at 2 h of exposure.

TABLE 3.3-1. METHYLENE CHLORIDE BLOOD CONCENTRATIONS DURING 4-h DERMAL VAPOR EXPOSURES TO RATS

	Methylene Chloride Concentration in Blood (μg/mL)[a]				
	Hours				
Concentration (ppm)	0.5	1.0	2.0	3.0	4.0
30,000	13.0	35.7	60.0	77.4	66.8
60,000	37.3	70.9	133.5	170.6	145.5
100,000	76.9	148.8	318.9	290.3	332.2

[a]Mean, N = 4 to 6.

Cannulated rats also were dermally exposed to a single concentration of dibromomethane (5000 ppm). A similar increase in blood concentration with steady-state values at about 2 h was noted (Table 3.3-2).

TABLE 3.3-2. DIBROMOMETHANE BLOOD CONCENTRATIONS DURING 4-h DERMAL VAPOR EXPOSURE TO RATS

	Dibromomethane Concentration in Blood (μg/mL)[a]				
	Hours				
Concentration (ppm)	0.5	1.0	2.0	3.0	4.0
5,000	12.4	27.9	52.5	81.9	84.6

[a]Mean, N = 5 to 6.

REFERENCES

AFOSH (Air Force Occupational Safety and Health) Standard 161-3, 20 June 1977, pp. 2-7.

Kinkead, E.R., R.E. Wolfe, S.A. Salins, and D.L. Pollard. 1992. Determination of blood concentrations following dermal exposure of chemical vapor. In: H. Wall, D. Dodd, A. Vinegar, and H. Clewell, eds. *1991 Toxic Hazards Research Unit Annual Report*, pp. 145-149. AL-TR-1992-0142, NMRI-92-97. Wright-Patterson Air Force Base, OH: Armstrong Laboratory and Naval Medical Research Institute.

McDougal, J.N., G.W. Jepson, H.J. Clewell III, and M.E. Andersen. 1985. Dermal absorption of dihalomethane vapors. *Toxicol. Appl. Pharmacol.* 79:150-158.

McDougal, J.N., G.W. Jepson, H.J. Clewell III, and M.E. Andersen. 1986. A physiological pharmacokinetic model for dermal absorption of vapors in the rat. *Toxicol. Appl. Pharmacol.* 85:286-294.

McDougal, J.N., G.W. Jepson, H.J. Clewell III, M.L. Gargas, and M.E. Andersen. 1990. Dermal absorption of organic chemical vapors in rats and humans. *Fundam. Appl. Toxicol.* 14:229-308.

3.4 METABOLISM OF VINYL CHLORIDE/TRICHLOROETHYLENE MIXTURES: PHARMACOKINETIC MODELING OF INHIBITION KINETICS IN RAT

H.A. Barton, J.R. Creech[1], C.S. Godin[2], G.M. Randall, and C.S. Seckel

ABSTRACT

Environmental and occupational exposures are typically to mixtures of chemicals, though most toxicity information is for individual compounds. Interactions between chemicals may involve pharmacokinetic and/or pharmacodynamic effects resulting in modulation of toxicity. Therefore, physiologically based pharmacokinetic modeling has been used to analyze data describing the metabolism of vinyl chloride (VC) and trichloroethylene (TCE) mixtures in rats. A single saturable pathway was modeled, representing cytochrome P_{450} 2E1. This was partially validated using preexposure to *trans*-1,2-dichloroethylene (tDCE). This virtually eliminated *in vivo* metabolism of both VC and TCE at low concentrations. Microsomes from tDCE-exposed animals showed inhibition of metabolism of P_{450} 2E1 substrates (chlorzoxazone, *p*-nitrophenol, and TCE) and no effect on 7-ethoxycoumarin deethylation. Studies with liver microsomes from VC-exposed animals found that neither suicide inhibition nor induction occurred during 6-h exposures to high concentrations. Therefore, these effects were not modeled. Modeling of mixtures of VC and TCE was successful only using competitive inhibition, as might be predicted for cytochrome P_{450} 2E1 substrates, and not uncompetitive or noncompetitive inhibition. These results were further validated by determining depletion of glutathione due to VC metabolism. The validation of a detailed model for the inhibition kinetics of metabolism of these two compounds permits better understanding of the implications of coexposures for toxicity. It is notable that competitive inhibition only becomes significant at relatively high concentrations, whereas at low concentrations, typical of environmental exposures, absorption is perfusion limited and enzyme is in excess so that the chemicals will be metabolized independently.

INTRODUCTION

Interactions between chemicals affecting their toxicity may result from alterations in pharmacokinetics (absorption, distribution, metabolism, or elimination) and/or pharmacodynamics (quantitative descriptions of the mechanisms of toxicity). Pharmacokinetic modeling is currently more completely developed than modeling

---

[1]Armstrong Laboratory, Toxicology Division, Wright-Patterson Air Force Base, OH.

[2]Current Address: Cephalon, Inc., West Chester, PA.

of toxicity processes. Therefore, pharmacokinetically important interactions are more readily characterized quantitatively.

Complex mixtures of organics and metals are typically present in contaminated surface and groundwater (Germolec et al., 1989). These mixtures arise from releases of the individual chemicals; environmental weathering, including biodegradation; and formation of by-products, for instance during chlorination. All three of these processes create mixtures of chloroethylenes. Chloroethylenes are particularly common contaminants due to their widespread usage as industrial chemicals. Trichloroethylene (TCE) and tetrachloroethylene are known to undergo anaerobic dechlorination in field and laboratory studies (Freedman and Gossett, 1989). This process results in the formation of TCE, dichloroethylene isomers, vinyl chloride (VC), and ethylene, though the extent of formation of each species is site-specific and variable.

Most animal studies of toxicity utilize individual pure chemicals, whereas human exposures and, consequently, epidemiological data mostly involve mixtures. The single chemical approach results from a scientific need to identify which chemicals in a mixture are of greatest concern. The problem of "reconstituting" mixtures is complicated by their infinite variation and the potential for complex interactions ranging from synergism and potentiation to antagonism (U.S. Environmental Protection Agency, 1986). The difficulties, particularly in the area of pharmacodynamics, are also increased greatly when the concern is chronic toxicity, such as cancer.

Vinyl chloride is a known human carcinogen that causes cancer, notably hepatoangiosarcomas, in every animal species tested (Agency for Toxic Substances and Disease Registry, 1989). Vinyl chloride is a classic mutagenic chemical due to formation of an electrophilic epoxide metabolite via metabolism by cytochrome P_{450} (P_{450}), particularly P_{450} 2E1 (Guengerich et al., 1991; Gwinner et al., 1983).

Chloroethylene oxide rearranges spontaneously to form chloroacetaldehyde (Gwinner et al., 1983). Chloroacetaldehyde is responsible for the majority of adduction of proteins. *In vitro* and *in vivo* studies have found P_{450} itself to be a target resulting in suicide inhibition (Guengerich and Strickland, 1977; Ivanetich et al., 1977; Pessayre et al., 1979a; Reynolds et al., 1975). Glutathione (GSH) depletion has been demonstrated *in vivo* and linked to chloroacetaldehyde formation (Plugge and Safe, 1977).

Trichloroethylene, by contrast, causes cancer in two animal species, but the results are highly species dependent (Bruckner et al., 1989). Oral exposure resulted in hepatocellular carcinomas in $B6C3F_1$ mice and some kidney tumors in male rats. Lung tumors also were seen in mice exposed by inhalation.

P_{450} 2E1 is the major enzyme responsible for the initial metabolism of TCE, though other P_{450}s can also be involved (Nakajima et al., 1992, 1993). Direct GSH-conjugation, mediated by transferases, also occurs at very low rates (Bruckner et al., 1989). The extent of epoxide formation appears limited, apparently

due to rearrangement occurring in the enzyme active site (Miller and Guengerich, 1983). Neither significant destruction of P_{450} nor GSH depletion have been reported for TCE. These results contrast strongly with those for VC but are similar to findings with tetrachloroethylene.

The studies reported here utilize *in vitro* measurements of enzyme activities and cofactor levels to validate the structure of a physiologically based pharmacokinetic (PBPK) model. A model previously developed for mixtures of TCE and 1,1-dichloroethylene (vinylidene chloride) was used with TCE and VC (Andersen et al., 1987). Competitive, noncompetitive, and uncompetitive inhibition of oxidative P_{450} metabolism were all considered as possible interactions between these chemicals. Differences in VC-induced GSH depletion assisted in better understanding the results of coexposures to TCE and VC. Finally, the implications of the results for risk assessment of mixtures were considered.

EXPERIMENTAL PROCEDURES

Animals

Male Sprague-Dawley rats were purchased from Charles River Breeding Laboratories (Raleigh, NC). Animals were quarantined for two weeks for verification of health status by pathological, fecal parisitological, and serum chemistry examinations. Water and food (Purina Formulab #5008) were provided *ad libitum*. On the day of experiments, body weights ranged from 150 to 500 g, though most weighed between 250 and 350 g. The animals used in this study were handled in accordance with the principles stated in the "Guide for the Care and Use of Laboratory Animals" prepared by the Committee on Care and Use of Laboratory Animal Resources, National Research Council, Department of Health and Human Services, National Institute of Health, Publication No. 86-23, 1985; and the Animal Welfare Act of 1966, as amended.

Chemicals

Chemicals were obtained from manufacturers with the specified purity: VC (Fluka, New York, NY, 99.5%), *trans*-1,2-dichloroethylene (tDCE) (Aldrich, Milwaukee, WI, 98%), and TCE (Aldrich, Milwaukee, WI, 99.9%). Purities were confirmed by gas chromatography (GC)/mass spectrometry. 6-Hydroxy-chlorzoxazone was obtained from the Toxicology Division, Wright-Patterson Air Force Base. All other chemicals were purchased from standard sources. Vinyl chloride is a known human carcinogen and appropriate precautions were taken to limit exposure of personnel to this gas. Trichloroethylene is a neurotoxin at high concentrations, so exposure to the liquid or its vapor was limited.

Gas Uptake Exposures

Three male Sprague-Dawley rats were exposed to VC, TCE, tDCE, or a mixture using a closed recirculating system similar to that described by Gargas et al. (1986). This method starts with a fixed amount of chemical in the atmosphere. The concentration decreases as the chemical is absorbed and metabolized. Chamber concentrations were monitored using a gas-sampling valve connected to a GC. Chromatography was performed on a 12 ft × 1/8 in. stainless steel column packed with 10% SE30 on 80/100 mesh Chromosorb WHP. The GC was equipped with a hydrogen flame ionization detector (FID) with the temperature set at 250 °C, an injection temperature of 175 °C, a constant oven temperature of 120 °C, and the nitrogen carrier at 21 cc/min. Maximum exposure time was 6 h.

Partition Coefficient Determinations

A modification of the vial equilibration method was used to determine partition coefficients (Gearhart et al., 1993). Animals were sacrificed by carbon dioxide (CO_2) asphyxiation. Blood was obtained from the posterior vena cava. Liver, fat, muscle (slow), and kidney (rapid) were collected for analysis. Tissues (except blood) were homogenized without diluent. Blood (1 mL) was added or tissues (1 g) were smeared on the side of a 21.9 mL vial. All samples were incubated for 3 h with VC (114 ppm) or TCE (600 ppm) before determining headspace concentration. Chromatographic conditions were the same as those for gas uptake.

Nonprotein Sulfhydryl

Nonprotein sulfhydryl (NPSH) was measured as an estimate of reduced GSH levels on livers following selected exposures (Ellman, 1959; Baker et al., 1990). Animals were sacrificed using CO_2. Livers were not perfused for NPSH analysis, but as much blood as possible was drained before mincing. Minced tissue was homogenized in five volumes of 5% sulfosalicylic acid. The supernatant (100 μL) from a 1500×g spin (10 min) was added to phosphate/ethylenediaminetetraacetic acid (80/0.8 mM final) and 5,5'-dithiobis-2-nitrobenzoic acid (0.15 mM final). Absorption at 412 nm was compared to GSH standard and was quantitated as moles GSH per milligram wet weight liver. The NPSH data (nmol GSH/mg protein) were analyzed using a one factorial analysis of variance with Tukey multiple comparisons (Rosner, 1990). The factor was exposure levels.

Induction of P_{450}s and Preparation of Microsomes

Induction of rats was carried out by intraperitoneal injection of pyridine (200 mg/kg) for 4 days or by addition of 10% ethanol or 0.1% phenobarbital to drinking water for 7 days. Livers from control and exposed

rats were removed and perfused with cold 0.15 M Tris-potassium chloride (KCl) (pH 7.4) with added heparin (2 U/mL). The liver was minced with four volumes of Tris-KCl and homogenized with a Tri-R-Stir-R tissue homogenizer Model K41 (Tri R Instruments, Rockville Centre, NY). The homogenate was centrifuged at $500\times g$ for 10 min at 4 °C and the supernatant was recentrifuged at $9000\times g$ for 10 min. The resulting supernatant was further centrifuged at $105{,}000\times g$ for 60 min. Microsomal pellets were suspended in 0.15 M Tris-KCl (pH 7.4) and then stored at -80 °C until use.

Assays for Enzymatic Activity

In vitro metabolism was measured by the formation of 6-hydroxychlorzoxazone using a modification of the procedure of Peter et al. (1990). Samples were injected onto a C18 reversed-phase column (4.6 mm × 150 mm) using a 25% acetonitrile/75% phosphoric acid (0.5%) mobile phase and a 15 min run time. 7-Ethoxycoumarin *O*-deethylation was measured using the procedure of Greenlee et al. (1978) modified to use a plate reader (CytoFluor 2300, Millipore Inc., Bedford, MA) with a fixed emission filter wavelength of 460 nm and excitation of 380 nm.

The TCE metabolism in rat microsomes was assessed by the disappearance of TCE from headspace using a modification of the method of Nakajima et al. (1990). The protein content was 1 to 2 mg in 1 mL 100 mM potassium phosphate (pH 7.4). Incubation time was 40 min at 37 °C after a 20 min preincubation time with TCE and no regenerating system. The regenerating system contained 0.66 mM nicotinamide-adenine dinucleotide phosphate, 13.7 mM glucose-6-phosphate, 2.8 U/mL glucose-6-phosphate dehydrogenase, and 100 mM potassium phosphate (pH 7.4). Experiments showed that the reaction was linear over this time period and the reduced nicotinamide-adenine dinucleotide phosphate regeneration was optimal. The TCE was added as a vapor. Headspace disappearance was measured by GC analysis on a Hewlett-Packard 5880 or 5890 equipped with an FID. A 10% SE 30 80/100 Chromasorb WHP 12' X 1/8" stainless steel column was used with the following conditions: oven temperature of 125 °C, detector temperature of 250 °C, injection temperature of 175 °C, and carrier flow of 21 cc/min nitrogen. The amount of TCE metabolized was determined using the calculations of Sato and Nakajima (1979).

Attempts to measure microsomal metabolism of VC *in vitro* by monitoring the disappearance of parent from vial headspace were unsuccessful, whereas good results were obtained with TCE. This made it impossible to perform experiments with VC/TCE mixtures to parallel the *in vivo* experiments.

PBPK Modeling

A modified version of a model for competitive, noncompetitive, and uncompetitive inhibition of enzyme metabolism by two substrates was used (Andersen et al., 1987). The model was run using SIMUSOLV® (The Dow Chemical Co, Midland, MI). This model consists of four physiological compartments: liver, fat, rapidly perfused (e.g., kidney), and slowly perfused (e.g., muscle) tissues. Metabolism was modeled as occurring entirely in the liver.

Parameter values other than chemical specific ones were: QPC (alveolar ventilation, L h^{-1} kg^{-1} = 21, QCC (cardiac output, L h^{-1} kg^{-1}) = 21; tissue blood flows as percent of cardiac output, QLC (liver) = 0.25, QFC (fat) = 0.09, QSC (slow) = 0.15, QRC (rapid) = 0.51; and tissue volumes as a fraction of body weight, VLC (liver) = 0.04, VFC (fat) = 0.07, VSC (slow) = 0.75, VRC (rapid) = 0.05 (Andersen et al., 1987). Measurements of cardiac output in Sprague-Dawley rats recently have been published from which QCC=21 was calculated (Delp et al., 1991). The values for QPC and QCC were set equal (i.e., QPC/QCC = 1) because the rats typically are inactive during the gas uptake exposures.

In this model, the Michaelis-Menten equation for metabolism of each compound was adapted as follows:

(1) $(VMAX1 \times CVL1)/(KM1 \times A1 + CVL1 \times A2)$

(2) $A1 = 1 + CVL2/KMI12 + (CVL2)^2/(KMI21 \times KM22)$

(3) $A2 = 1 + CVL2/KM21 + CVL1/KM11$

where VMAX1 and KM1 represent the apparent maximal rate and concentration, respectively, producing half-maximal activity for Chemical 1; CVL1 and CVL2 are the venous concentrations of Chemicals 1 and 2, respectively, in blood leaving the liver, the only metabolizing organ in the model; and KMI12 is the apparent constant for inhibition of metabolism of Chemical 1 by Chemical 2 and similarly, KMI21, KM21, KM11, and KM22 are apparent inhibition constants where the first number indicates the chemical being metabolized and the second number is the chemical acting as inhibitor.

When the appropriate inhibitory constants are set to a large number (10^6), these equations reduce to the standard equations for the three types of inhibition. Values for competitive inhibition were KMI12=KM1, KMI21=KM2, and KM12=KM21=KM11=KM22=1,000,000. Values for noncompetitive inhibition were KMI12=0.65, KMI21=2.2, KM12=0.65, KM21=2.2, and KM11=KM22=1,000,000. Values for uncompetitive inhibition were KM12=3.0, KM21=3.0, and KMI12=KMI21=KM11=KM22=1,000,000.

RESULTS

Estimating Metabolic Parameters for VC with a Single Saturable Oxidative Pathway

Modeling VC pharmacokinetics requires chemical-specific parameters describing its partitioning into tissues (Table 3.4-1) and its metabolism. At a minimum, a single saturable pathway representing P_{450} oxidation is required. *In vitro* studies to validate this single saturable pathway were then undertaken. Initial concentrations in the gas uptake studies ranged from 95 to 10,000 ppm (Figure 3.4-1). Optimization of individual runs (n = 18) using QPC=QCC=21 estimated $Vmax_c$ = 3.8 ± 1.3 mg/kg·h (61 μmol/kg·h) and Km = 0.34 ± 0.19 mg/L (5 μM).[3]

TABLE 3.4-1. PARTITION COEFFICIENTS FOR VINYL CHLORIDE AND TRICHLOROETHYLENE

	Vinyl Chloride	
Blood/Air (PB1)	2.4±0.5	(n = 7)
Fat/Blood (PF1)	10.0±3.0	(5)
Muscle/Blood (PS1)	0.4±0.2	(4)
Liver/Blood (PL1)	0.7±0.3	(5)
Kidney/Blood (PR1)	0.7±0.4	(6)
	Trichloroethylene	
Blood/Air (PB2)	20.5±1.4	(n = 4)
Fat/Blood (PF2)	26.0±5.0	(3)
Muscle/Blood (PS2)	0.6±0.1	(8)
Liver/Blood (PL2)	1.3±0.1	(6)
Kidney/Blood (PR2)	1.0±0.2	(5)

Liver NPSH After VC Exposure

Depletion of NPSH, largely GSH, occurs during exposure to VC (Watanabe et al., 1976). Therefore, this biological effect of VC metabolism was used to validate some aspects of the model and demonstrate, by correlation, that altered VC metabolism was occurring as predicted by the model (Tables 3.4-2, -3, and -4).

[3]Values reported here and throughout this report are mean ± standard deviation.

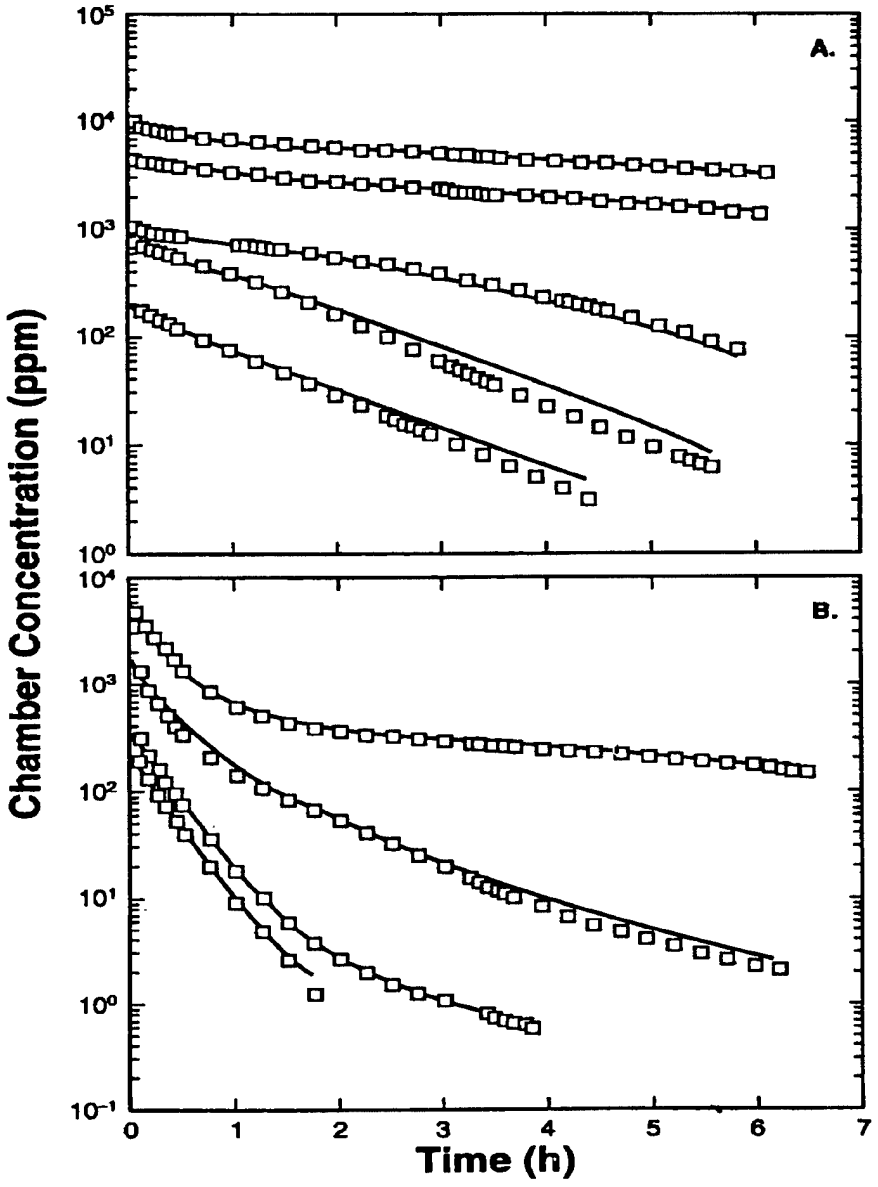


Figure 3.4-1. Metabolism of Individual Chemicals. (A) Vinyl chloride (□) chamber concentrations decrease as it is metabolized by rats. Solid lines represent model simulations of data. (B) Trichloroethylene (□) concentrations measured in the chamber. Solid lines represent model simulations of data.

TABLE 3.4-2. NONPROTEIN SULFHYDRYL (NPSH) CONTENT IN LIVERS OF RATS EXPOSED FOR 6 h TO VINYL CHLORIDE (VC), TRICHLOROETHYLENE (TCE), OR MIXTURES

Exposures (6 h)	Initial Concentration (ppm)	NPSH (nmol/mg)	NPSH Depletion (%)	Replicates:Rats
Untreated	-	5.5±0.6	-	30:6
VC	200	5.8±0.3	0	15:3
	600	5.2±0.5	5	14:3
	1000	4.1±0.6[a,b,c]	25	45:9
	5000	3.0±0.5[a,b,c,d]	44	24:6
TCE	600	5.5±0.8	0	15:3
	1000	5.7±0.7	0	15:3
	5000	5.4±0.9	0	15:3
VC/TCE Mixtures	5000:600	3.6±0.4[a]	35	15:3
	5000:5000	4.4±0.4[a]	20	30:6
	1000:1000	4.3±0.3[a]	22	29:6
	600:5000	5.2±0.3	5	15:3
	35:1000	5.9±0.4	0	15:3

[a] Significantly different than 0 ppm control (6 h) at $p<0.01$.
[b] Significantly different than 200 ppm (6 h) at $p<0.01$.
[c] Significantly different than 600 ppm (6 h) at $p<0.01$.
[d] Significantly different than 1000 ppm (6 h) at $p<0.01$.

TABLE 3.4-3. NONPROTEIN SULFHYDRYL (NPSH) IN RATS EXPOSED TO VINYL CHLORIDE (VC) AND *trans*-1,2-DICHLOROETHYLENE (tDCE)

Exposure	Initial Concentration (ppm)	NPSH (nmol/mg)	NPSH depletion (%)	Replicates:Rats
Untreated	-	6.0±1.0[a]	-	43:17
VC (3 h)	600	4.9±0.5[b]	18	16:6
	5000	4.0±0.9[b]	34	4:2
tDCE (4.5 h)	40	6.4±0.8	0	6:3
tDCE:VC (1.5:3 h)	40:200	5.9±0.4	0	10:5
	40:5000	5.5±0.6	0	16:8

[a] Not significantly different than 0 ppm control (6 h) at $p<0.01$.
[b] Significantly different than 0 ppm control (3 h) at $p<0.01$.

TABLE 3.4-4. CORRELATION OF MODELED VINYL CHLORIDE (VC) METABOLISM WITH MEASURED NONPROTEIN SULFHYDRYL (NPSH) DEPLETION

Starting Concentration (ppm)	NPSH Depletion Measured after 6 h (%)	Modeled VC Metabolized (μmol)
600 VC	5[a]	53
1000 VC	25	77
5000 VC	44	121
5000 VC/600 Trichloroethylene (TCE)	35	104
5000 VC/5000 TCE	20	79
1000 VC/1000 TCE	21	75

[a] This value represents partial recovery because depletion after 3 h was 18%.

Modulation of P_{450} Levels Potentially Requiring Modeling

Alterations in enzyme levels may occur through several mechanisms, including suicide inhibition, altered gene expression (induction or repression), and a variety of posttranscriptional alterations such as reduced turnover due to stabilization of protein. All three of these effects are known to occur for P_{450} 2E1 (Yang et al., 1990) and other P_{450} isoforms.

Enzymatic activities using several substrates were measured in liver microsomes from control and VC-exposed animals. The animals were exposed to a starting concentration of 10,000 ppm (1%) or 5000 ppm VC for a 6-h period, during which the chamber atmosphere was monitored. At these high (saturating) concentrations, the exposures more closely approximate constant concentration exposures.

Chlorzoxazone (25 μM) hydroxylation and TCE metabolism *in vitro* were measured as indicators of P_{450} 2E1 activity (Tables 3.4-5 and -6). No difference in activity was seen between animals treated with high VC concentrations and untreated controls. Microsomes from pyridine and ethanol-treated animals were used as positive controls and showed about fivefold increase in activity as expected. Deethylation of 7-ethoxycoumarin was also apparently unaffected by a 6-h exposure to a high concentration of VC (Table 3.4-7). By contrast, a significant increase in activity was seen with phenobarbital induction.

Together, these results show no significant effect of high VC treatment on selected P_{450} activities, suggesting that no effects would occur at low VC concentrations either. Thus, protein stabilization, enzyme induction, and suicide inhibition were not modeled.

TABLE 3.4-5. CHLORZOXAZONE (25 μM) METABOLISM BY RAT LIVER MICROSOMES

Treatment	Rate (nmol/min/mg) ± SD (Percent of Control Value)	Replicates:Rats
Unexposed controls	0.060±0.018	29:10
10,000 ppm vinyl chloride (VC)[a]	0.054±0.016	20:3
5000 ppm VC[a]	0.055±0.012	12:5
40 ppm *trans*-1,2-dichloroethylene (tDCE) (1.5 h)[a]	0.025±0.012[b] (42%)	6:3
40 ppm tDCE (4.5 h)[a]	0.025±0.011[b] (42%)	6:3
Pyridine induced	0.37±0.08[b] (617%)	6:3
Ethanol induced	0.27±0.03[b] (450%)	3:3

[a] Nominal starting concentrations in closed-chamber experiments.

[b] Statistically different than controls at $p < 0.01$.

TABLE 3.4-6. *IN VITRO* TRICHLOROETHYLENE METABOLISM BY RAT LIVER MICROSOMES

Treatment	Rate (nmol/min/mg) ± SD (Percent of Control Value)	Replicates:Rats
Unexposed controls	0.70±0.20	62:12
10,000 ppm vinyl chloride (VC) (6 h)[a]	0.74±0.22	8:3
5000 ppm VC (6 h)[a]	0.72±0.18	15:5
40 ppm *trans*-1,2-dichloroethylene (tDCE) (1.5 h)[a]	0.21±0.07[b] (39%)	9:3
40 ppm tDCE (4.5 h)[a]	0.11±0.05[b] (16%)	7:3
40 ppm tDCE (1.5 h) & 200 ppm VC (3 h)[a]	0.07±0.02[b] (10%)	12:3
Pyridine induced	1.7±0.1[b] (243%)	5:3

[a] Nominal starting concentrations in closed-chamber experiments.

[b] Statistically different than controls at $p < 0.01$.

TABLE 3-4.7. 7-ETHOXYCOUMARIN DEETHYLATION BY RAT LIVER MICROSOMES

Treatment	Rate (nmol/min/mg) ± SD (Percent of Control Value)	Replicates:Rats
Unexposed controls	1.3±0.4	57:11
10,000 ppm vinyl chloride (6 h)[a]	1.1±0.2	15:3
40 ppm *trans*-1,2-dichloroethylene (tDCE) (1.5 h)[a]	1.7±0.1[b] (133%)	3:2
40 ppm tDCE (4.5 h)[a]	1.7±0.1[b] (132%)	8:3
Phenobarbital induced	5.1±0.5[b] (392%)	3:3

[a] Nominal starting concentrations in closed-chamber experiments.

[b] Statistically different than controls at $p < 0.01$.

In Vivo Demonstration of P_{450} 2E1 Metabolism of VC

The involvement of P_{450} 2E1 in VC metabolism *in vivo* was demonstrated using tDCE. *trans*-1,2-Dichloroethylene has been suggested to be a suicide inhibitor based upon limited *in vitro* studies (Costa and Ivanetich, 1982) and modeling of gas uptake results (Gargas et al., 1990).

In vitro chlorzoxazone hydroxylation (Table 3.4-5) and *p*-nitrophenol metabolism (data not shown) were reduced by half after 1.5 and 4.5 h of tDCE exposure (40 ppm initial concentration) (Table 3.4-5). The tDCE blocked the metabolism of TCE *in vitro* between 61 and 84% (Table 3.4-6). Deethylation of 7-ethoxycoumarin, in contrast, showed a small increase (Table 3.4-7), indicating P_{450} 2E1 catalyzed reactivity was reduced with some specificity.

Animals were exposed to tDCE for 90 min (40 ppm starting concentration) prior to the addition of VC. The VC was added directly into the chamber so both chemicals were present for the remainder of the exposure. Metabolism of VC (200 ppm starting concentration) during 3 h was decreased approximately 85% (Figure 3.4-2). Liver NPSH was unaffected by exposure to tDCE alone or followed by 200 ppm VC (Table 3.4-3). Microsomes prepared from these animals showed a 90% decrease for *in vitro* TCE metabolizing capability, in good agreement with the *in vivo* results.

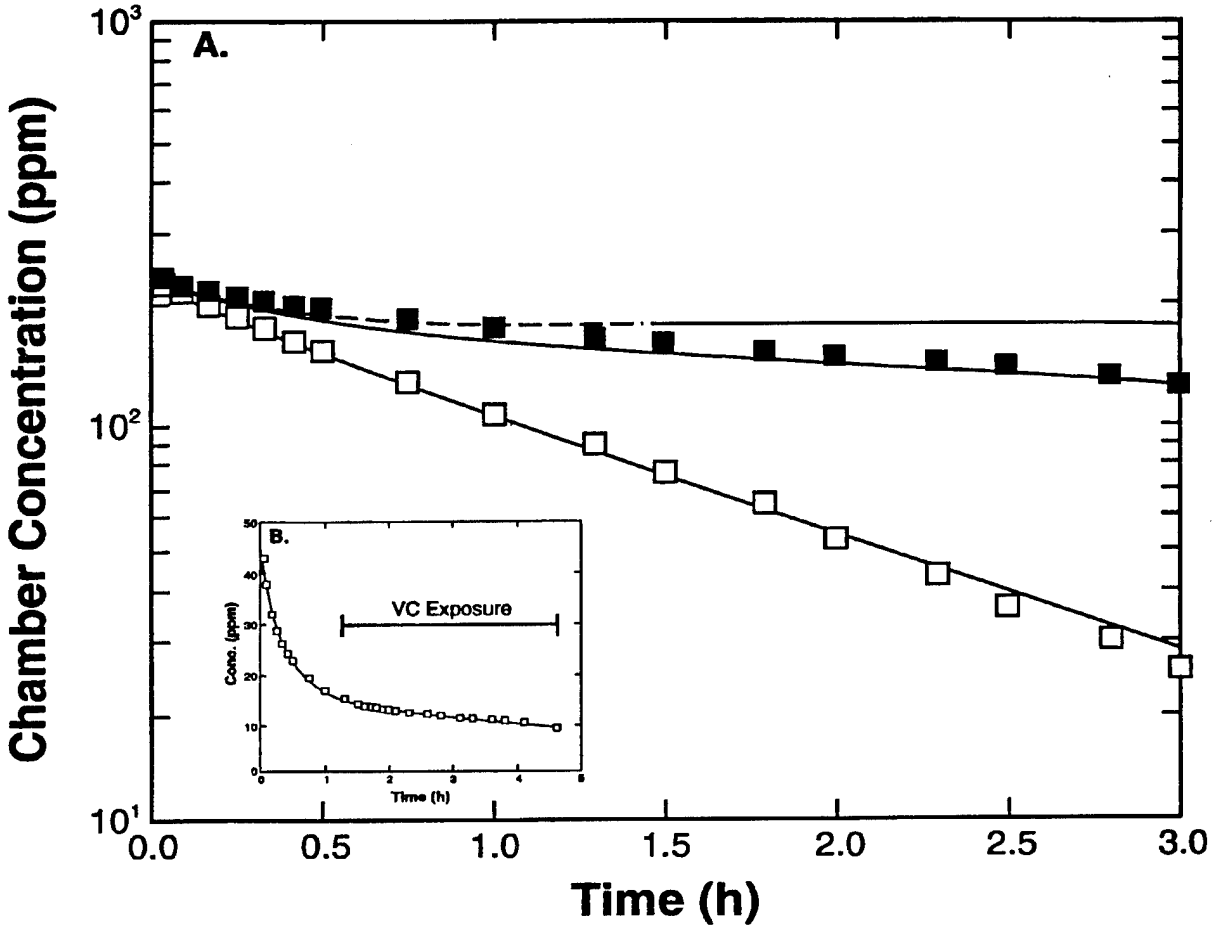


Figure 3.4-2. Metabolism of VC in the Presence and Absence of tDCE. (A) The decrease in VC chamber concentration is much greater in the absence (□) than in the presence of (■) of tDCE. Solid lines represent model simulations. The dashed line is modeled VC concentrations with no metabolism ($Vmax_c$=0). (B) The tDCE exposure started 1.5 h prior to the addition of VC. The effects of tDCE cannot be accounted for by competitive inhibition alone.

Estimating Metabolic Parameters for TCE

Partition coefficients were determined *in vitro* for blood, liver, fat, kidney, and muscle from male Sprague-Dawley rats (Table 3.4-1). These values were used in the PBPK model to fit gas uptake data for TCE and estimate an apparent Km and $Vmax_c$ (Figure 3.4-1). Optimization of six individual gas uptake exposures (200 to 6500 ppm) estimated Km = 0.44 ± 0.32 mg/L (approximately 3 μM) and $Vmax_c$ = 9.5 ± 2.6 mg/kg·h (72 μmol/kg·h).

Liver NPSH after TCE

Depletion of NPSH, largely GSH, does not occur to any significant extent following TCE metabolism (Table 3.4-2).

Effect of tDCE Preexposure on TCE Metabolism

Preexposure of rats to approximately 40 ppm tDCE largely inhibited TCE metabolism at initial concentrations, though there was some apparent concentration dependence. Exposure to an initial concentration of 200 ppm showed 95% inhibition ($Vmax_c$ = 0.5 mg/kg·h). Exposures starting at 500 and 2000 ppm showed 71% and 76% inhibition, respectively ($Vmax_c$ = 2.6 and 2.1 mg/kg·h). These data support the involvement of P_{450} 2E1 as the major form of P_{450} involved in TCE metabolism even at relatively high concentrations, although some involvement of other isoforms, as suggested by *in vitro* data (Nakajima et al., 1992), cannot be excluded.

Gas Uptake with Mixtures of VC and TCE

Gas uptake exposures were performed using several different combinations of VC and TCE. These initial results were used in the PBPK model to estimate the various inhibitory constants required for competitive, noncompetitive, and uncompetitive inhibition. Using these parameters, the model was run to simulate potential results given different exposure concentrations. This gave estimates of concentrations that would likely distinguish between the three forms of inhibition.

Metabolic parameters were estimated for 15 mixtures using the competitive inhibition model. The average values were Km = 0.10 ± 0.01 mg/L (1 μM) and $Vmax_c$ = 3.4 ± 0.4 mg/kg·h (45 μmol/kg·h) for VC. The TCE kinetic constants were estimated to be Km = 0.30 ± 0.06 mg/L (1 μM) and $Vmax_c$ = 9.3 ± 1.4 mg/kg·h (66 μmol/kg·h). These values are consistent with those estimated for the individual chemicals, although the standard deviations are much smaller, particularly for Km.

The gas uptake exposures to a mixture and the simulated results are illustrated in Figure 3.4-3. These data were used to distinguish between competitive, noncompetitive, and uncompetitive inhibitory kinetics. It should be noted that mixtures of high concentrations of both chemicals (e.g., 5000 ppm of both VC and TCE) were uninformative for distinguishing kinetic mechanisms.

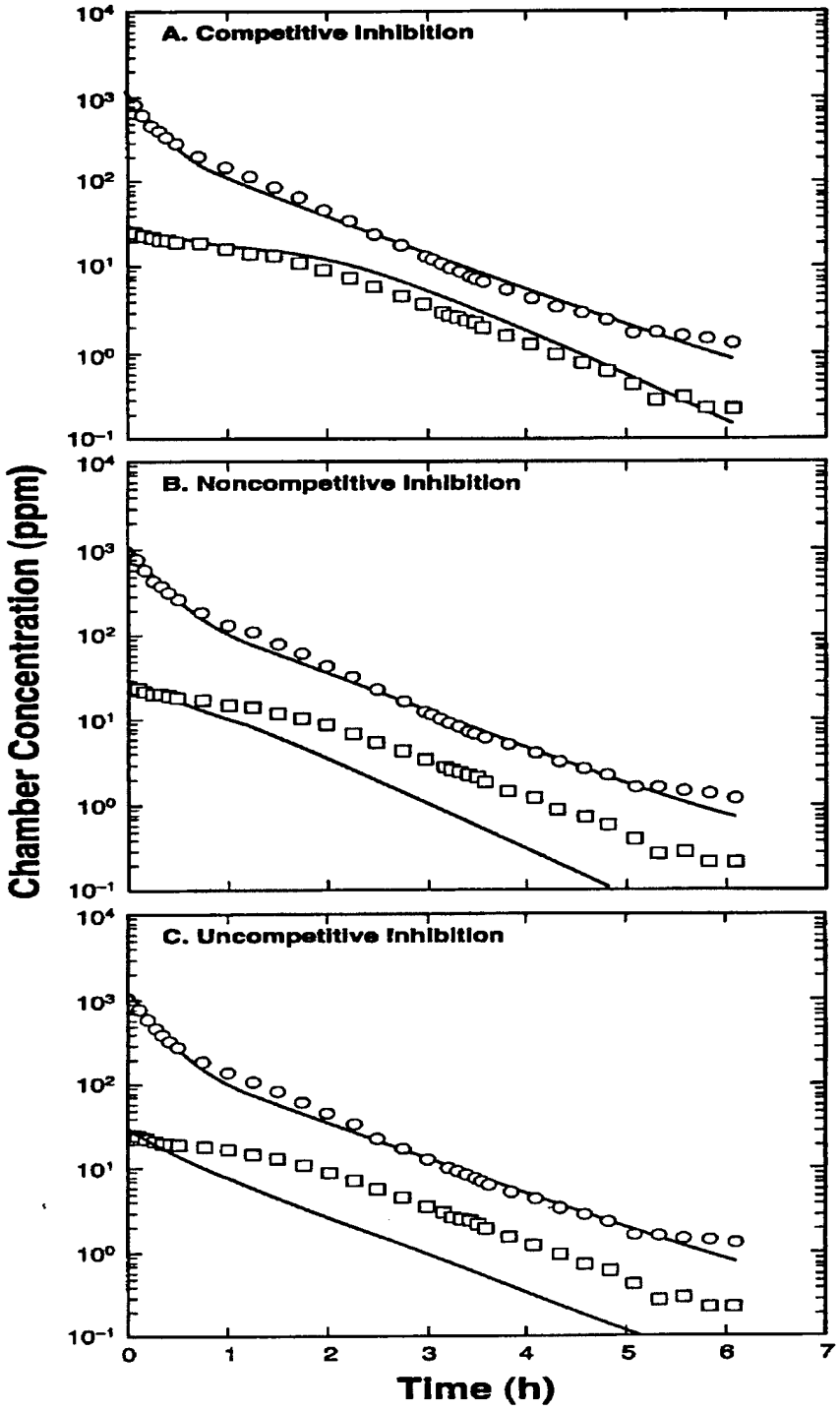


Figure 3.4-3. A Mixture with Initial Concentration of 30 ppm VC (□) and 1000 ppm TCE (○). Data (□,○) and simulation (solid line). (A) Competitive inhibition. (B) Noncompetitive inhibition. (C) Uncompetitive inhibition. Only competitive inhibition gives a reasonable fit to the data using parameters listed in Table 3.4-2.

Figure 3.4-3 illustrates the process for 30 ppm VC and 1000 ppm TCE. Noncompetitive inhibition was easily excluded (Figure 3.4-3b). Optimization of each individual mixture for the inhibition constants of the noncompetitive model resulted in estimates of KMI12 and KMI21 varying 100-fold. Thus, it was impossible to use a single set of parameters for noncompetitive inhibition to consistently fit all mixtures data. Uncompetitive inhibition was more difficult to rule out. For many of the mixtures, a reasonable simulation of gas uptake data was obtained using a single set of constants describing uncompetitive inhibition. However, this provided a poor fit for a few mixtures (e.g., 30:1000, Figure 3.4-3c). It was possible to adjust the parameters for uncompetitive inhibition to simulate the mixture of 30:1000, but then the other mixtures could not be simulated successfully. The mixture of 60:1000 gave similar results. Thus, uncompetitive inhibition does not provide satisfactory simulation of all the mixtures using a single consistent set of parameters.

Validation of Mixtures Modeling with NPSH Measurements

Livers of animals exposed to mixtures of TCE and VC were removed and analyzed for NPSH. Increasing the concentration of TCE (600 to 5000 ppm starting concentration) present simultaneously with 5000 ppm VC resulted in less NPSH depletion, 35% and 20%, respectively (Table 3.4-4) than in the absence of TCE, 44%.

DISCUSSION

Modeling was begun with the assumption that a single saturable pathway representing P_{450} 2E1 in the liver compartment might be adequate to describe initial oxidative metabolism of VC and TCE. However, suicide inhibition via formation of a heme adduct by VC or related vinyl halogens has previously been demonstrated (Reynolds et al., 1975; Ortiz de Montellano et al., 1982). P_{450} 2E1 is also frequently "induced" by exposure to its substrates, although the mechanisms may not be transcriptional (Yang et al., 1990). Finally, limited *in vitro* data suggested that VC is metabolized by several P_{450} isoforms (Peyssayre et al., 1979a), and extensive studies show this to be true for TCE (Nakajima et al., 1992). Modeling mixtures requires having good models for the individual chemicals, so these aspects were investigated further. Choices of physiological parameters also can be important, so a comparison of two different values for cardiac output (and ventilation rate) was made for each individual chemical and the mixtures.

Suicide inhibition of P_{450} by VC has been demonstrated both *in vitro* and *in vivo* at high concentrations (about 5% or 50,000 ppm) using untreated and phenobarbital-induced animals (Ivanetich et al., 1977; Reynolds et al., 1975). To determine if this effect needed to be modeled, metabolism of several P_{450} substrates was measured in liver microsomes from rats exposed to approximately 2000 or 5000 ppm VC. No differences

were found between control and VC-treated rats in metabolism of chlorzoxazone, TCE, or 7-ethoxycoumarin. Because suicide inhibition is a stochastic occurrence, these data indicate it occurs too rarely to be apparent *in vivo* at concentrations used in chronic studies (≤10,000 ppm) with VC. No evidence exists for such effects with TCE (Pessayre et al., 1979b). Therefore, suicide inhibition was not modeled for either compound.

These studies also provide no evidence for increased P_{450} activity due to exposure to VC or TCE for 6 h at high concentrations. The P_{450} 2E1 activity is increased by both transcriptional and posttranscriptional regulation, including protein stabilization by substrate (Yang et al., 1990). No such effects were apparent using *in vitro* assays and microsomes from VC-exposed animals. The *in vivo* data for both VC and TCE were consistent with a constant Vmax. Whether changes might occur during chronic exposures, such as during cancer studies, has not been determined.

Finally the issue of modeling P_{450} isoforms was considered. This issue is important for extrapolation from high to low doses and across species using PBPK models as is required for cancer risk assessment.

Based upon existing literature, one would expect P_{450} 2E1 to metabolize both TCE and VC (Guengerich et al., 1991; Nakajima et al., 1993). However, monoclonal antibodies to P_{450} 2E1 only inhibited 60% of the TCE metabolism in control microsomes (at their low concentration) (Nakajima et al., 1992). P_{450} 2B1 also metabolizes TCE, but is present at very low levels in untreated rats, so its involvement would be expected to be minimal. Trichloroethylene also is metabolized by the male rat specific isoform P_{450} 2C11 *in vitro*.

trans-1,2-Dichloroethylene exposure at moderate concentrations was a highly effective inhibitor of the metabolism of the other chlorinated ethylenes. Quantitatively, the inhibition of TCE metabolism was similar *in vitro* and *in vivo*. *In vitro* assays of enzyme activity with microsomes from the tDCE-treated rats clearly show large decreases in other activities associated with P_{450} 2E1 (chlorzoxazone, *p*-nitrophenol). Essentially no change was seen in 7-ethoxycoumarin activity by comparison. These results show that P_{450} 2E1 activity accounts for much of the TCE and VC metabolism *in vivo* with untreated animals.

Coexposures to VC and TCE are successfully described as competitive inhibition using a fixed single saturable pathway for oxidative metabolism. A wide range of mixtures were used in gas uptake studies in order to distinguish between kinetic inhibition models. Competitive inhibition consistently simulated the data using a single set of parameters. Furthermore, the kinetic parameter values calculated from the competitive inhibition model are essentially identical to those obtained from studies with individual chemicals. By contrast, noncompetitive inhibition would fit very few data sets with a single set of parameter values. Uncompetitive inhibition tended to more readily simulate multiple data sets, except for several key mixtures that were only fit with widely varying parameter values.

The results of the mixtures simulations was further validated using GSH depletion. This end point was chosen because it was dependent upon metabolism of VC. Glutathione conjugation is an important pathway for detoxification of VC metabolites. This is apparently only an acute effect because GSH levels return to control values as exposures continue over a period of days (Du et al., 1979). The potential implications of this aspect for modeling VC carcinogenicity remain to be explored.

Finally, the results of this study are of interest from the perspective of risk assessment for mixtures. Exposure of humans to chlorinated ethylene mixtures may occur occupationally, generally by inhalation. Environmental exposure may be oral or by inhalation. Exposure to water may involve both oral and inhalation pathways, the latter due to water uses such as washing or showering. The inhalation studies reported here may readily be extrapolated to the oral route using the PBPK model.

Although one might expect P_{450} 2E1 substrates to be competitive inhibitors in mixtures, such effects are highly concentration dependent. The inhibition effects observed here only occur at relatively high concentrations, as has also been reported for competitive inhibition between toluene and xylene (Tardif et al., 1993). Occupational exposures to chlorinated hydrocarbons may be as high as low parts per million, but environmental exposures are more typically at low parts-per-billion levels. These latter are clearly in a range where the metabolism becomes perfusion limited and enzyme is saturating, so no inhibition would be predicted to occur. Under these conditions, the pharmacokinetics of the compounds would be independent. This is consistent with the standard U.S. Environmental Protection Agency approach of assuming additivity. However, it remains to be shown that the pharmacodynamics of the two chemicals also are independent. Toxicity, and thus chemical risk assessment, ultimately depends upon both pharmacokinetics and pharmacodynamics.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the expert assistance of Carlyle Flemming for the statistical analyses and Joanne Drerup for her laboratory work.

REFERENCES

Andersen, M.E., M.L. Gargas, H.J. Clewell III, and K.M. Severyn. 1987. Quantitative evaluation of the metabolic interactions between trichloroethylene and 1,1-dichloroethylene *in vivo* using gas uptake methods. *Toxicol. Appl. Pharmacol.* 89:149-157.

Agency for Toxic Substances and Disease Registry. 1989. Toxicological profile for vinyl chloride. ATSDR/TP-88/25.

Baker, M.A., G.J. Cerniglia, and A. Zaman. 1990. Microtiter plate assay for the measurement of glutathione and glutathione disulfide in large numbers of biological samples. *Anal. Biochem.* 190:360-365.

Bruckner, J.V., B.D. Davis, and J.N. Blancato. 1989. Metabolism, toxicity, and carcinogenicity of trichloroethylene. *Crit. Rev. Toxicol.* 20:31-50.

Costa, A.K. and K.M. Ivanetich. 1982. The 1,2-dichloroethylenes: Their metabolism by hepatic cytochrome P-450 *in vitro*. *Biochem. Pharmacol.* 31:2093-2102.

Delp, M.E., R.O. Manning, J.V. Bruckner, and R.B. Armstrong. 1991. Distribution of cardiac output during diurnal changes of activity in rats. *Am. J. Physiol.* 261:H1487-H1493.

Du, J.T., J.P. Sandoz, M.T. Tseng, and C.H Tamburro. 1979. Biochemical alterations in livers of rats exposed to vinyl chloride. *J. Toxicol. Environ. Health* 5:1119-1132.

Ellman, G.L. 1959. Tissue sulfhydryl groups. *Arch. Biochem. Biophys.* 82:70-77.

Freedman, D.L. and J.M. Gossett. 1989. Biological reductive dechlorination of tetrachloroethylene and trichloroethylene to ethylene under methanogenic conditions. *Appl. Environ. Microb.* 55:2144-2151.

Gargas, M.L., M.E. Andersen, and H.J. Clewell III. 1986. A physiologically based simulation approach for determining metabolic constants from gas uptake data. *Toxicol. Appl. Pharmacol.* 86:341-352.

Gargas, M.L., H.J. Clewell III, and M.E. Andersen. 1990. Gas uptake inhalation techniques and the rates of metabolism of chloromethanes, chloroethanes, and chloroethylenes in the rat. *Inhal. Toxicol.* 2:295-319.

Gearhart, J.M., D.A. Mahle, R.J. Greene, C.S. Seckel, C.D. Flemming, J.W. Fisher, and H.J. Clewell III. 1993. Variability of physiologically based pharmacokinetic PBPK model parameters and their effects on PBPK model predictions in a risk assessment for perchloroethylene PCE. *Toxicol. Lett.* 68:131-144.

Germolec, D.R., R.S.H. Yang, M.F. Ackermann, G.J. Rosenthal, G.A. Boorman, P. Blair, and M.I. Luster. 1989. Toxicology studies of a chemical mixture of 25 groundwater contaminants: II. Immunosuppression in B6C3F1 mice. *Fundam. Appl. Toxicol.* 13:377-387.

Greenlee, W.F. and A. Poland. 1978. An improved assay of 7-ethoxycoumarin *O*-deethylase activity: Induction of hepatic enzyme activity in C57BL/6J and DBA/2J mice by phenobarbital, 3-methylcholanthrene, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *J. Pharmacol. Exper. Ther.* 205:596-605.

Guengerich, F.P. and T.W. Strickland. 1977. Metabolism of vinyl chloride: Destruction of the heme of highly purified liver microsomal cytochrome P-450 by a metabolite. *Molec. Pharmacol.* 13:993-1004.

Guengerich, F.P., D-H. Kim, and M. Iwasaki. 1991. Role of human cytochrome P-450 IIE1 in the oxidation of many low molecular weight cancer suspects. *Chem. Res. Toxicol.* 4:168-179.

Gwinner, L.M., R.J. Laib, J.G. Filser, and H.M. Bolt. 1983. Evidence of chloroethylene oxide being the reactive metabolite of vinyl chloride towards DNA: Comparative studies with 2,2′-dichloro-diethylether. *Carcinogenesis* 4:1483-1486.

Ivanetich, K.M., I. Aronson, and I.D. Katz. 1977. The interaction of vinyl chloride with rat hepatic microsomal cytochrome P-450 *in vitro*. *Biochem. Biophys. Res. Comm.* 74:1411-1418.

Miller, R.E. and F.P. Guengerich. 1983. Metabolism of trichloroethylene in isolated hepatocytes, microsomes, and reconstituted enzyme systems containing cytochrome P-450. *Cancer Res.* 43:1145-1152.

Nakajima, T., R-S. Wang, N. Murayama, and A. Sato. 1990. Three forms of trichloroethylene-metabolizing enzymes in rat liver induced by ethanol, phenobarbital, and 3-methylcholanthrene. *Toxicol. Appl. Pharmacol.* 102:546-552.

Nakajima, T., R-S. Wang, E. Elovaara, S.S. Park, H.V. Gelboin, and H. Vainio. 1993. Cytochrome P-450-related differences between rats and mice in the metabolism of benzene, toluene and trichloroethylene in liver microsomes. *Biochem. Pharmacol.* 45:1079-1085.

Nakajima, T., R-S. Wang, E. Elovaara, S.S. Park, H.V. Gelboin, and H. Vainio. 1992. A comparative study on the contribution of cytochrome P-450 isozymes to metabolism of benzene, toluene, and trichloroethylene in rat liver. *Biochem. Pharmacol.* 43:251-257.

Ortiz de Montellano, P.R., K.L. Kunze, H.S. Beilan, and C. Wheeler. 1982. Destruction of cytochrome P-450 by vinyl fluoride, fluroxene, and acetylene. Evidence for a radical intermediate in olefin oxidation. *Biochemistry* 21:1331-1339.

Pessayre, D., J.C. Wandscheer, V. Descatoire, J.Y. Artigou, and J.P. Benhamou. 1979a. Formation and inactivation of a chemically reactive metabolite of vinyl chloride. *Toxicol. Appl. Pharmacol.* 49:505-515.

Pessayre, D., H. Allemand, J.C. Wandscheer, V. Descatoire, J-Y. Artigou, and J-P. Benhamou. 1979b. Inhibition, activation, destruction, and induction of drug-metabolizing enzymes by trichloroethylene. *Toxicol. Appl. Pharmacol.* 49:355-363.

Peter, R., R. Bocker, P.H. Beaune, M. Iwasaki, F.P. Guengerich, and C.S. Yang. 1990. Hydroxylation of chlorzoxazone as a specific probe for human liver cytochrome P-450IIE1. *Chem. Res. Toxicol.* 3:566-573.

Plugge, H. and S. Safe. 1977. Vinyl chloride metabolism — A review. *Chemosphere* 6:309-325.

Reynolds, E.S., M.T. Moslen, S. Szabo, and R.J. Jaeger. 1975. Vinyl chloride-induced deactivation of cytochrome P-450 and other components of the liver mixed function oxidase system: An *in vivo* study. *Res. Comm. Chem. Path. Pharmacol.* 12:685-694.

Rosner, B. 1990. *Fundamentals of Biostatistics*. Boston, MA: PWS-Kent Publishing Company.

Sato, A. and T. Nakajima. 1979. A vial-equilibration method to evaluate the drug-metabolizing enzyme activity for volatile hydrocarbons. *Toxicol. Appl. Pharmacol.* 47:41-46.

Tardif, R., S. Lapare, K. Krishnan, and J. Brodeur. 1993. Physiologically based modeling of the toxicokinetic interaction between toluene and *m*-xylene in the rat. *Toxicol. Appl. Pharmacol.* 120:266-273.

U.S. Environmental Protection Agency. 1986. Guidelines for the health risk assessment of chemical mixtures. 51 FR 34014.

Watanabe, P.G., R.E. Hefner, and P.J. Gehring. 1976. Vinyl chloride-induced depression of hepatic non-protein sulfhydryl content and effects on bromosulphalein BSP clearance in rats. *Toxicology* 6:1-8.

Yang, C.S., J-S.H. Yoo, H. Ishizaki, and J. Hong. 1990. Cytochrome P-450 IIE1: Roles in nitrosamine metabolism and mechanisms of regulation. *Drug Metab. Rev.* 22:147-159.

3.5 TETRACHLOROETHYLENE: EXPOSURE AND RISK ASSESSMENTS IN BREAST-FED INFANTS

J.Z. Byczkowski and J.W.Fisher[1]

ABSTRACT

Tetrachloroethylene (also known as perchloroethylene; PCE) is a volatile, nonflammable liquid widely used in the dry cleaning industry and in metal degreasing operations. If inhaled by a lactating woman, PCE may partition to breast milk and may be transferred to the breast-fed infant. Recently, Schreiber (1992) described a summary of an exposure and risk assessment of PCE in human breast milk, employing a four-compartment physiologically based pharmacokinetic (PBPK) model without a milk compartment. Altering this model, Schreiber (1992) estimated the relationship between PCE concentration in the mother's breathing zone and in the breast milk, assuming that human milk is an adipose tissue containing 4% fat. Using this estimate, she assessed the excess cancer risk for infants exposed to PCE in breast milk. In contrast to this approach, we have developed and experimentally validated a PBPK model for lactational transfer of PCE in rats, including a quantitative description of the milk compartment and the nursing pup (Byczkowski et al., 1993). Subsequently, our model has been scaled to human physiology (Byczkowski and Fisher, 1994) and was validated with human literature data for PCE exposure cases. The model predictions were in good agreement with both the measured values and those reported in the literature. In the present paper, we have applied our model to scenarios described by Schreiber (1992) for occupationally and nonoccupationally exposed mothers, simulating PCE concentrations in breast milk and the infant exposure. Our predictions gave similar magnitude of infant exposures as those estimated by Schreiber and, thus, suggested similar cancer risks associated with these exposures. Comparison of the predictions generated with various models confirms the usefulness of PBPK modeling in risk assessments.

INTRODUCTION

Tetrachloroethylene (also known as perchloroethylene; PCE) is a volatile, nonflammable liquid widely used in dry cleaning and metal degreasing. Acute inhalation of PCE vapor by humans has produced central nervous system depression ranging from lightheadedness and muscular incoordination to loss of consciousness and respiratory paralysis (Stewart et al., 1961a, 1970; Stewart, 1969). A minor, reversible hepatic

[1] Toxicology Division, Occupational and Environmental Health Directorate, Armstrong Laboratory, Wright-Patterson Air Force Base, OH.

dysfunction has been noted several days following the accidental human exposure to anesthetic concentrations of PCE (Stewart, 1969; Stewart et al., 1970). Under chronic exposure, PCE is suspected to cause cancer (Greenberg and Parker, 1983). Widespread applications of PCE generate noticeable air concentrations of this volatile chemical not only in occupational facilities, but also in some residential settings, especially those adjacent to the dry cleaning services (New York State Department of Health, 1991). One clinical case of lactational transfer of PCE was described by Bagnell and Ellenberger (1977). In this case, substantial concentrations of PCE (up to 10 ppm) were detected in milk from a Canadian woman who regularly visited her husband during his lunch hour at a dry cleaning factory. Her nursing infant developed obstructive jaundice at age 6 weeks, but recovered quickly after cessation of breast-feeding. The authors attributed the disease to the contamination of breast milk with PCE (Bagnell and Ellenberger, 1977); however, it is still not clear whether the association of the disease with exposure to PCE was causal or spurious.

Several reports in the literature have described pharmacokinetics of PCE in mice, rats and humans (reviewed in Ward et al., 1988). The authors also described a four-compartment physiologically based pharmacokinetic (PBPK) model for the pharmacokinetics of PCE in male rats and mice following PCE inhalation exposures (Ward et al., 1988). Subsequently, Schreiber (1992) used this four-compartment model to estimate human breast milk concentrations of PCE and to assess the excess cancer risk for infants exposed to PCE in breast milk. However, the nursing infant assessment by Schreiber (1992) was based on solubility of PCE in fat, but not in milk.

In contrast to this approach, we have developed and experimentally validated a PBPK model for lactational transfer of PCE in rats, including a quantitative description of the milk compartment and the nursing pup (Byczkowski et al., 1993). Recently, our model has been scaled to reflect human physiology (Byczkowski and Fisher, 1994) and was validated with human literature data for PCE exposure cases. The model predictions were in good agreement with both the measured values and those reported in the literature. In the present paper, we have applied our model to scenarios described by Schreiber (1992) for occupationally and nonoccupationally exposed mothers, simulating PCE concentrations in breast milk as well as the infant exposure.

A PBPK MODEL DESCRIPTION

A PBPK model was written in SIMUSOLV, a FORTRAN-based continuous simulation language, and simulations were performed using a SIMUSOLV software package with optimization capabilities (DOW Chemical Co., Midland, MI) on a VAX/VMS mainframe computer (VAX8530, Digital Equipment Corp., Maynard, MA). Parameters were optimized by SIMUSOLV, which is using the log likelihood function as the

criterion and either the generalized reduced gradient method for single parameter optimization or the Nelder-Mead search method for multiple parameters optimization to adjust the values.

Figure 3.5-1 shows the scheme of the PBPK model developed in nursing rats, essentially as described by Ramsey and Andersen (1984). Additional compartments were added to describe milk (Shelley et al., 1988) and nursing pups (Byczkowski et al., 1993). Initially, for simplicity, the pups were described by the lungs, arterial and venous blood, and the "other tissues" compartment (Byczkowski et al., 1993). However, this simplified model did not adequately describe the kinetics of PCE in pup blood and tissues. Thus, milk was retained in the gastrointestinal tract of nursing pups, apparently causing delayed absorption of PCE for several hours. To describe this phenomenon, an additional compartment was added simulating the pup gastrointestinal tract. Subsequently, the model was scaled-up to describe human physiology (Figure 3.5-1).

THE DIFFERENTIAL EQUATIONS

Mass transfer differential equations describing each compartment building the PBPK model for lactational transfer of PCE (schematically shown in Figure 3.5-1) are described below.

For well-stirred compartments without metabolism or other losses (fat tissue, slowly perfused and rapidly perfused tissues, pup tissues), the amount change (dA*i*) over time was described as follows:

$$dAi/dt = Qi(CA - CVi)$$

where subscript *i* represents *i*th compartment, Q*i* represents the blood flow through the *i*th compartment, CA represents the arterial concentration, and CV*i* represents the venous concentration leaving the *i*th compartment (CV*i* = C*i*/P*i*, where C*i* is a concentration in the tissue in *i*th compartment and P*i* is the tissue/blood partition coefficient for *i*th compartment; and C*i* = A*i*/V*i*, where A*i* represents the amount in the *i*th compartment and V*i* represents the volume of the *i*th compartment).

For the liver compartment, a loss term (RAM) was added to the well-stirred compartment description to account for metabolism (RAM = VMAX×CVL/(KM + CVL) + KF×CVL×VL; where VMAX is the pseudo-maximal velocity rate of PCE metabolism, CVL is the venous concentration leaving the liver, KM is the apparent Michaelis-Menten constant, KF is the first order rate of metabolism, and VL is the volume of the liver compartment):

$$dAL/dt = QL(CA - CVL) - RAM$$

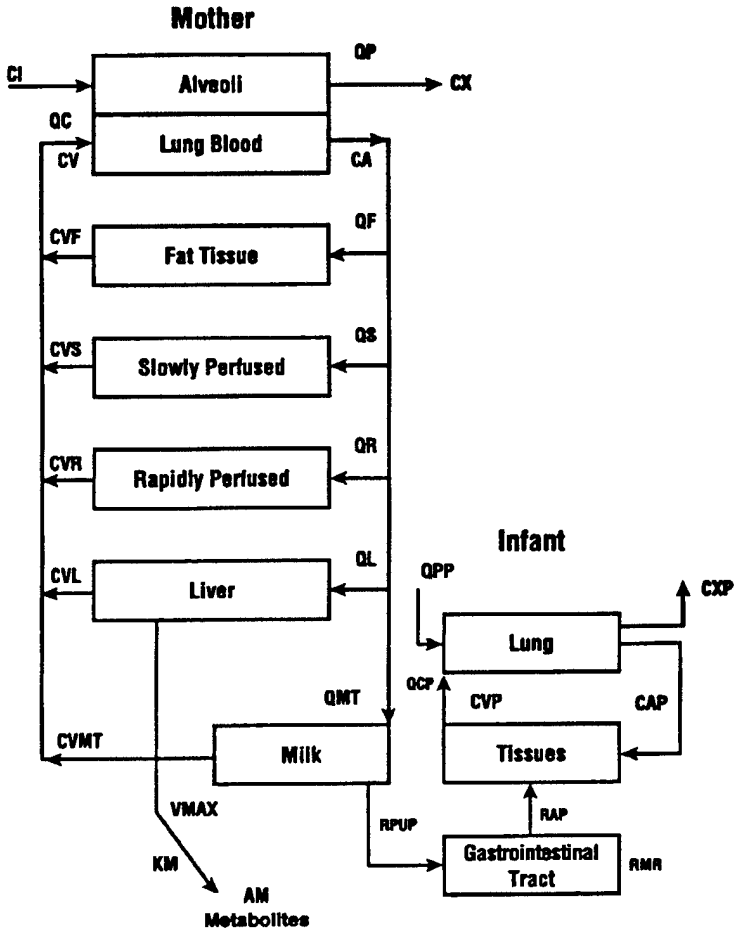


Figure 3.5-1. Scheme of PBPK Model Used to Simulate Lactational Transfer of PCE in Nursing Rats (Byczkowski et al., 1993) and Humans (Byczkowski and Fisher, 1994).

Abbreviations: CI=concentration in inhaled air (mg/L); QP=alveolar ventilation rate adjusted for body weight (L/h); CX=concentration in exhaled air (mg/L); QC=cardiac output adjusted for body weight (L/h); CVF=venous concentration leaving the fat tissue (mg/L); QF=blood flow to fat (L/h); CVS=venous concentration leaving the slowly perfused tissues (mg/L); QS=blood flow to slowly perfused tissues (L/h); CV=concentration in mixed venous blood (mg/L); CA=concentration in arterial blood (mg/L); CVR=venous concentration leaving the rapidly perfused tissues (mg/L); QR=blood flow to rapidly perfused tissues (L/h); CVL=venous concentration leaving the liver tissue (mg/L); QL=blood flow to liver (L/h); QPP=alveolar ventilation rate in infant adjusted for body weight (L/h); CXP=concentration in air exhaled by infant (mg/L); CVMT=venous concentration leaving the mammary glands tissue (mg/L); CMAT=concentration in milk (mg/L); QCP=cardiac output in infant adjusted for body weight (L/h); CVP=concentration in venous blood in infant (mg/L); CAP=concentration in arterial blood in infant (mg/L); RPUP=elimination rate for PCE from milk to infant (mg/h); VMAX=pseudo-maximal velocity of PCE metabolism (mg/h); KM=apparent Michaelis-Menten constant for PCE metabolism (mg/L); AM=amount of PCE metabolized (mg); RMR=the rate of gastrointestinal tract loading with PCE in infant (mg/h): RAP=the rate of gastrointestinal absorption of PCE in infant (mg/h).

Analogously, for the mammary glands compartment, the change of amount (dAMAT), described as above, contained a loss term for elimination of PCE from milk to pups, RPUP (RPUP = CMAT×OUTX, where CMAT is the concentration in milk and OUTX is a periodic zero-order milk yield per dam such that OUTX = OUTI or is set to OUTX = 0 for a "no feeding" period):

$$dAMAT/dt = QMT(CA - CVMT) - RPUP$$

where QMT represents mammary blood flow and CVMT represents the venous concentration leaving the mammary glands (CMAT = AMAT/VMILK, where CMAT is the concentration in the mammary glands and VMILK represents the volume of milk). It was assumed that the milk compartment is in intimate contact with the arterial blood perfusing the mammary tissue, and that PCE rapidly equilibrates with the milk.

The rate of change in the amount of PCE in the pup gastrointestinal tract (AGIT) was described as a difference between the rate of ingesting of PCE with mother's milk (RPUP) and the rate of absorption from the gastrointestinal tract, RAP (RAP = MR×KAP, where MR is the amount remaining in the pup gastrointestinal tract and KAP is the absorption constant for the pup, determined to be equal to 0.5 h^{-1}):

$$dAGIT/dt = RPUP - RAP$$

The concentration of PCE in the pup gastrointestinal tract (CGIT) was calculated as CGIT = MR/GIW, where GIW represents the weight of the gastrointestinal tract of the pup, adjusted for the pup's weight.

COMPUTER SIMULATIONS AND PREDICTIONS OF PCE DISTRIBUTION IN HUMANS

The PBPK model was scaled up and its predictions were tested versus available data for humans. The blood/air and milk/air partition coefficients for PCE were measured by our laboratory in the samples collected from volunteer donors (Fisher et al., 1993). Initially, a set of physiological parameters and kinetic constants pertinent to PCE in humans was adopted from Ward et al. (1988). The other values describing human milk, infant, and mammary glands compartments were calculated from data published for "Reference Man" (International Commission on Radiological Protection, 1984), and finally the constants were optimized using the SIMUSOLV software package, over the experimental data from the literature describing inhalation exposures of human subjects to PCE (Fernandez et al., 1976; Bolanowska and Golacka, 1972; Stewart et al., 1961b, 1970). The final set of parameters and constants is listed in Table 3.5-1.

TABLE 3.5-1. KINETIC CONSTANTS AND PHYSIOLOGICAL PARAMETERS USED IN PBPK MODELING OF LACTATIONAL TRANSFER OF PCE IN HUMANS

Description			[Units]
Tissue Volumes	[Fraction of Body Weight:BW]		
Maternal			
Liver	VLC	=	0.04
Fat	VFC	=	0.2
Mammary	VMATC	=	0.05
Neonatal			
Infant Tissue	VTCP	=	0.9
	[L]		
Maternal			
Slowly Perfused	VS	=	0.79×BW-VF-VMAT
Rapidly Perfused	VR	=	0.12×BW-VL
Milk Volume	VMILK	=	0.04375
Flow Rates	[L/h/kg]		
Maternal			
Alveolar Ventilation	QPC	=	19.7
Cardiac Output	QCC	=	18.0
Neonatal			
Alveolar Ventilation Infant	QPCP	=	25.2
Cardiac Output Infant	QCCP	=	22.0
	[Fraction of Cardiac Output]		
Maternal			
Liver	QLC	=	0.25
Fat	QFC	=	0.05
Partition Coefficients	[Ratio of Solubility]		
Maternal			
Blood/Air	PB	=	19.8
Liver/Blood	PL	=	6.83
Fat/Blood	PF	=	159.03
Slowly Perfused/Blood	PS	=	7.77
Rapidly Perfused/Blood	PR	=	6.83
Milk/Blood	PMILK	=	2.8
Neonatal			
Blood/Air Infant	PPB	=	8.0
Other Tissue/Blood Infant	PPT	=	6.596
Metabolism			
Maternal			
	[mg/L]		
Apparent Michaelis-Menten	KM	=	0.32
	[mg/kg/h]		
Pseudo Maximal Velocity	VMAXC	=	0.151

Using these parameters, with the milk compartment turned off, and adopting the exposure scenario described by the American Conference of Governmental Industrial Hygienists (ACGIH) (1992), the computer simulations of PCE concentrations in human blood and exhaled air were run and then compared to the published Biological Exposure Indices values. The model only slightly underpredicted both blood and exhaled air PCE concentrations for human subjects, prior to the last shift of the workweek (Byczkowski and Fisher, 1994). A much better fit of the computer-simulated time-course was obtained with the data reported by Fernandez et al., 1976) for exhaled air of human subjects exposed to 100 ppm of PCE for 1 and 8 h (Byczkowski and Fisher, 1994). Similarly, the model accurately predicted PCE concentrations in exhaled air of human subjects exposed to 194 ppm of PCE, reported by Stewart et al. (1970). The model predictions of PCE exhaled breath clearance rates were also in general agreement with values measured by Bolanowska and Golacka (1972), with modest overprediction of experimental data after the first measured time point. However, the model predictions fit better to the experimental data from the slim man than from the obese woman (Byczkowski and Fisher, 1994).

COMPUTER SIMULATIONS AND PREDICTIONS OF PCE DISTRIBUTION IN A MOTHER AND HER NURSING INFANT

We also have simulated the only documented case of the lactational transfer of PCE from mother to infant, described by Bagnell and Ellenberger (1977). Although the PCE concentration in inhaled air was not measured, the reported incidents of dizziness after exposure of mother to PCE without symptoms of general anesthesia (Bagnell, personal communication) suggested a PCE air concentration within the range of several hundred parts per million. The best approximation of the computer-simulated values to PCE concentrations determined in breast milk by Bagnell and Ellenberger (1977) was achieved when the exposure concentration in inhaled air was assumed to be 600 parts per million (Figure 3.5-2). This concentration, exceeding more than 10 times the air threshold limit value (TLV)-time-weighted average level recommended by ACGIH (1992) for PCE, could result in the infant blood concentration of not more than 0.035 mg/L within 1 month of exposure to PCE via mother's milk, reaching a total daily infant dose of 0.91 mg PCE/kg/day (Byczkowski and Fisher, 1994). Although our estimate was lower than the 1.4 mg PCE/kg/day estimated by Schreiber (1992), the infant blood concentrations were in both cases more than one order of magnitude lower than the no-effect threshold assumed for adults by ACGIH (1992). It is, however, unclear if they could cause adverse health effects in infants.

From these several predictions of PCE distribution and its kinetic behavior in exhaled air, blood and milk of exposed human subjects, and especially from the comparison of computer simulations with the available human data from literature, it was concluded that the PBPK model successfully describes PCE concentrations in both lactating rats and humans. Consequently, this validated PBPK model was used to predict the absorbed doses of PCE by the nursing infant from the concentrations in mother's breathing zone and the indoor PCE in a residential setting, as described by Schreiber (1992).

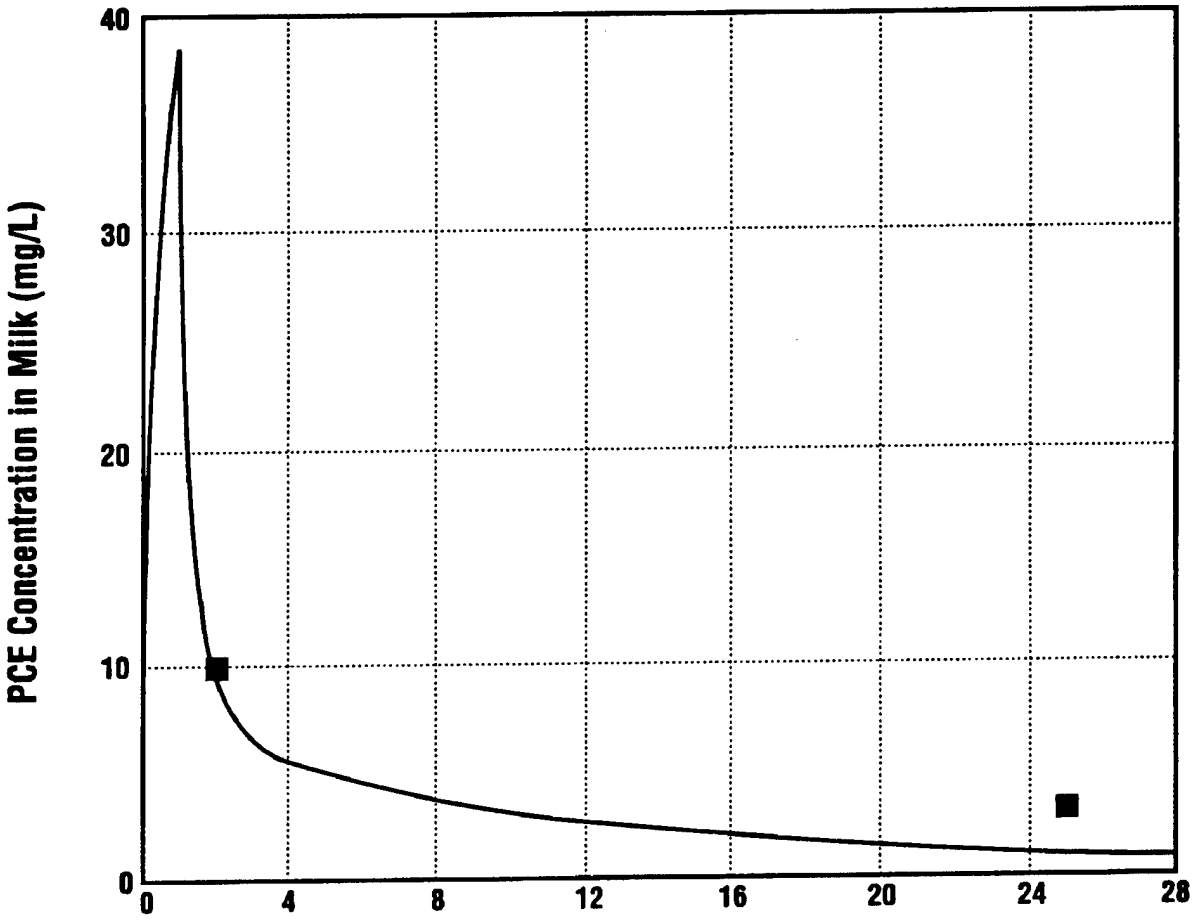


Figure 3.5-2. Computer Simulation (solid line) of Time-Dependent Concentrations of PCE in Milk of Lactating Mother Assumed to Be Exposed to 600 ppm of PCE for 1 h, According to the Scenario Reported by Bagnell and Ellenberger (1977). Small rectangles show data measured in milk of human subject according to Bagnell and Ellenberger (1977).

COMPUTER SIMULATIONS OF SEVEN EXPOSURE SCENARIOS

It was assumed after Schreiber (1992) that a 7.2 kg infant ingests 0.7 L of breast milk per day. The following seven exposure scenarios were adapted after Schreiber (1992).

A. Occupationally exposed mother inhaling air containing PCE at the ACGIH TLV of 50 ppm (340 mg/m^3), 8 h per day, 5 days per week, followed by exposure to an indoor residential background concentration (IRBC) of 0.0041 ppm (IRBC = 0.028 mg/m^3, according to the New York State Department of Health [NYDH], 1991).

B. Occupationally exposed mother inhaling air containing PCE at the Occupational Safty and Health Administration permissible exposure limit of 25 ppm (170 mg/m^3), 8 h per day, 5 days per week, followed by exposure to an IRBC of 0.0041 ppm.

C. Occupationally exposed mother inhaling air containing 5.9 ppm (40 mg/m^3, approximate arithmetic mean concentration for counter-workers, pressers, and

seamstresses), 8 h per day, 5 days per week, followed by exposure to an IRBC of 0.0041 ppm.

D. Nonoccupationally exposed mother inhaling air containing PCE of 6.7 ppm (45.8 mg/m^3, the 24-h average concentration reported by NYDH [1991] in an apartment located above a dry cleaner using an old dry-to-dry machine), 7 days per week.

E. Nonoccupationally exposed mother inhaling air containing PCE of 1.1 ppm (7.7 mg/m^3, 24-h average concentration reported by NYDH [1991] in apartments located above dry cleaners using transfer machines), 7 days per week.

F. Nonoccupationally exposed mother inhaling air containing PCE of 0.037 ppm (0.25 mg/m^3, 24-h average reported by NYDH [1991] above dry cleaners using dry-to-dry machines), 7 days per week.

G. Nonoccupationally exposed mother inhaling air containing PCE at IRBC of 0.0041 ppm, 7 days per week.

The results of computer simulation of exposure scenario A (solid lines) are compared in Figure 3.5-3 with maximum simulated concentrations in blood and milk (a, b), daily infant dose from milk (c), and total daily infant dose (d) estimated by Schreiber (1992) (small rectangles). Our simulation included 10 weeks of exposure (T=1680 h), until the daily infant dosage reached the plateau (Figure 3.5-3, c and d).

It seems, that although the maximum simulated concentrations in blood (CV = 1.32 mg/L) estimated by Schreiber (1992) were much lower than our prediction (up to 3 mg/L, Figure 3.5-3a), the concentrations in milk (CMAT) matched perfectly well (CMAT = 8.5 mg/L, Figure 3.5-3b). On the other hand, both daily infant dose from milk (IDM, Figure 3.5-3c) and total daily infant dose (TOTINF, Figure 3.5-3d) were estimated by Schreiber (1992) to be more than twice the levels predicted by our simulation (0.82 mg/kg/day versus 0.34 mg/kg/day and 0.82 mg/kg/day versus 0.33 mg/kg/day, respectively).

This discrepancy was not unexpected, as Schreiber (1992) according to the "generic" PBPK model has assumed that the infant is fed by the mother with breast milk containing always the peak concentrations of PCE. According to our computer simulation, the mother started feeding her infant when PCE CMAT was falling rapidly after the end of an 8-h shift of the occupational exposure. Also during weekends, the PCE concentrations in breast milk dropped even farther (Figure 3.5-3b). Similarly, the IDMs were overpredicted by Schreiber (1992) in Scenarios B (Figure 3.5-4a), C (Figure 3.5-4b), D (Figure 3.5-4c), E (Figure 3.5-4d), and F (Figure 3.5-4e) as compared to our simulation. The differences between the estimate by Schreiber (1992) and our computer simulation of daily IDM were negligible in scenario G, after 8 weeks of maternal exposure (Figure 3.5-4f).

The TOTINFs were further overpredicted by Schreiber (1992) in Scenarios B (Figure 3.5-5a) and C (Figure 3.5-5b) as compared to our simulation, where the occupational maternal PCE exposure for 8 h was the main source of infant exposure via breast milk. In contrast to those, the TOTINFs were roughly twice underpredicted by Schreiber (1992) in Scenarios D (Figure 3.5-5c), E (Figure 3.5-5d), F (Figure 3.5-5e), and G (Figure 3.5-5f) as compared to our simulation, where the sources of the daily dosage in the infant were the residential maternal PCE exposure along with residential infant PCE exposure, both for 24 h.

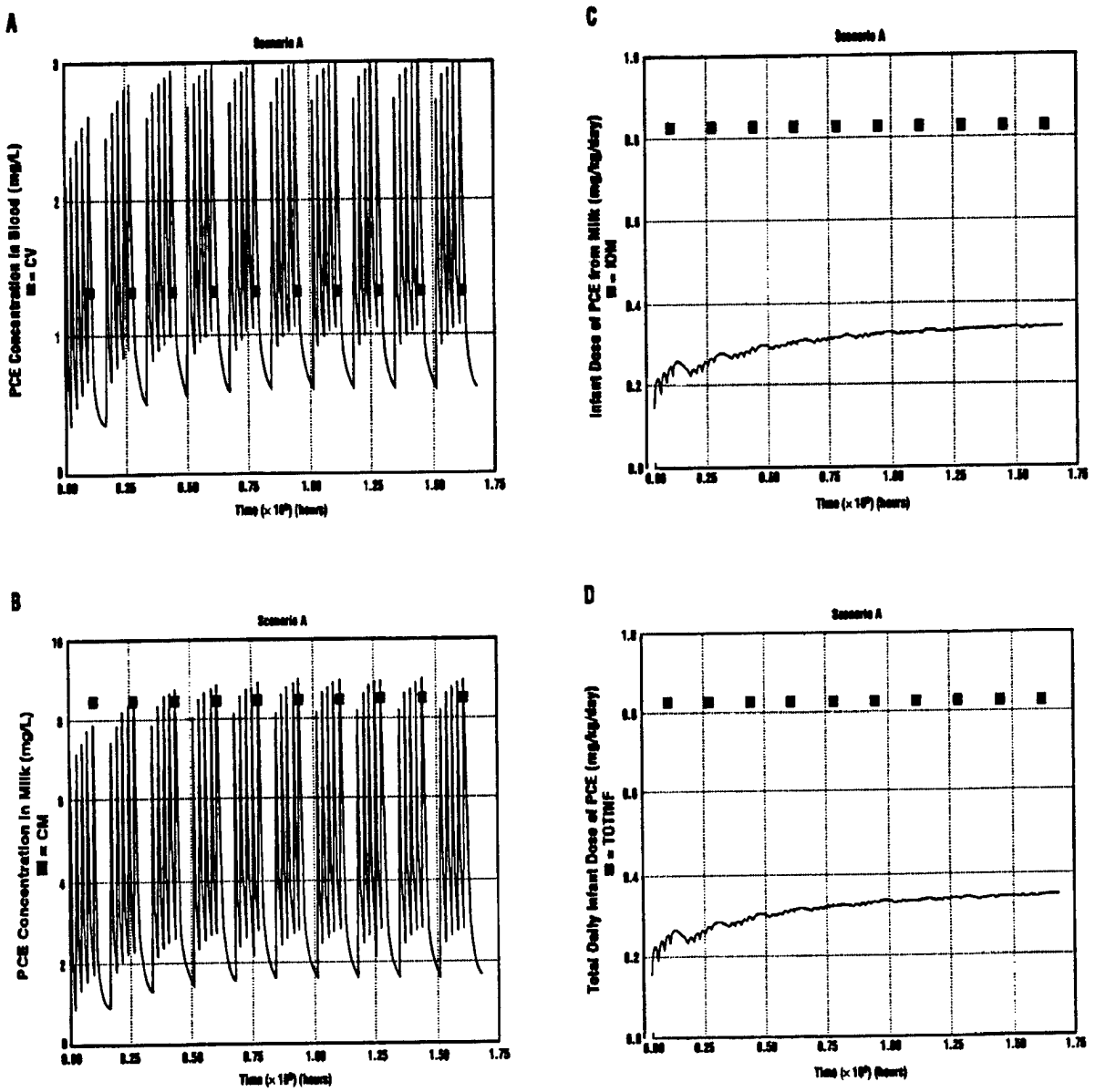


Figure 3.5-3. Computer Simulations (solid lines) of Time-Dependent Concentrations of PCE in Blood (A) and Milk (B) of Lactating Mother, Daily Infant Doses of PCE from Milk (C), and Total Daily Infant Doses of PCE (D) According to the Exposure Scenario A (Schreiber, 1992). Small rectangles show the level of maximum simulated concentrations in blood and milk (A, B), daily infant dose from milk (C), and total daily infant dose (D) estimated by Schreiber (1992).

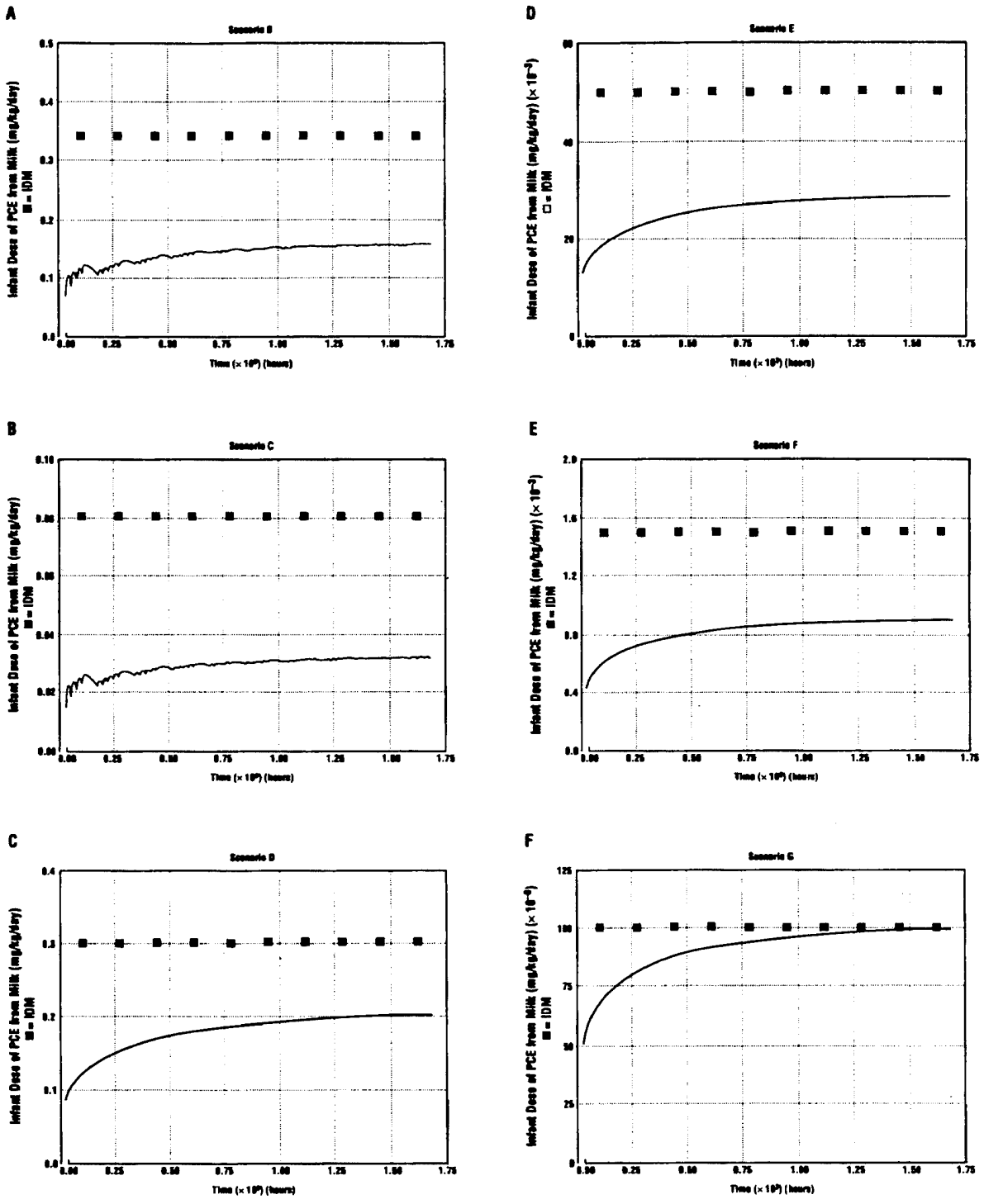


Figure 3.5-4. Computer Simulations (solid lines) of Time-Dependent Daily Infant Doses of PCE from Milk According to Different Exposure Scenarios (Schreiber, 1992). Small rectangles show the level of daily infant doses from milk estimated by Schreiber (1992).

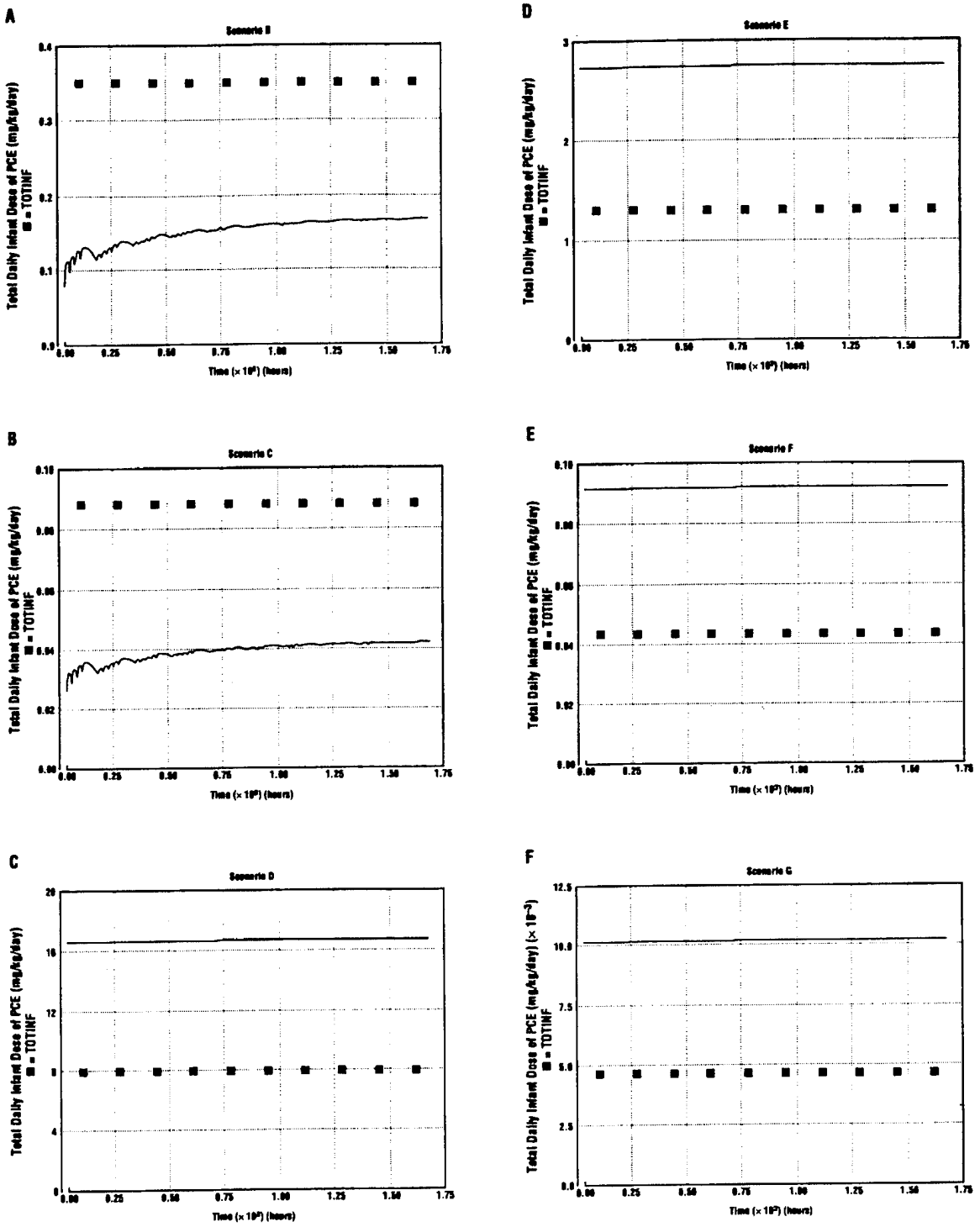


Figure 3.5-5. Computer Simulations (solid lines) of Time-Dependent Total Daily Infant Doses of PCE According to Different Exposure Scenarios (Schreiber, 1992). Small rectangles show the level of total daily infant doses estimated by Schreiber (1992).

CANCER RISK ASSESSMENT IN INFANTS

The estimated PCE IDMs and TOTINFs listed in Table 3.5-2 were used for excess and total risk assessment for infants.

TABLE 3.5-2. THE ESTIMATED EXPOSURE AND EXCESS CANCER RISK ASSESSMENT FOR INFANTS[a]

	IDM	TOTINF	ECRI		
Scenario	(mg/kg/day)		Schreiber (1992)	This Model	TCRI
A	0.333	0.344	6.0E-4	2.4E-4	2.5E-4
B	0.156	0.167	2.5E-4	1.1E-4	1.2E-4
C	0.032	0.042	5.8E-5	2.3E-5	3.1E-5
D	0.202	16.669	2.2E-4	1.5E-4	1.2E-2
E	0.029	2.732	3.6E-5	2.1E-5	2.0E-3
F	0.0009	0.092	1.4E-6	6.6E-7	6.7E-5
G	0.0001	0.010	1.0E-7	7.3E-8	7.3E-6

[a] IDM = infant dose from milk, TOTINF = total daily exposure infant dose (ingested + inhaled), ECRI = excess cancer risk from drinking contaminated breast milk, TCRI = total theoretical infant cancer risk from PCE (ingested + inhaled).

The excess cancer risk estimates for infants (ECRIs) were calculated as follows:

$$ECRI = 5.1E\text{-}2 \times IDM \times 1/70$$

where IDM is infant dose from milk (mg/kg/day). It was assumed, after Schreiber (1992), that the infant may be exposed to PCE in breast milk for 1 year of a 70-year lifetime. An oral potency factor of PCE was assumed to be 0.051 mg/kg/day, according to U.S. Environmental Protection Agency (EPA) cancer risk methodology for PCE (Schreiber, 1992).

The total cancer risk estimates for infants (TCRIs) were calculated as follows:

$$TCRI = 5.1E\text{-}2 \times TOTINF \times 1/70$$

where TOTINF is daily exposure infant dose (mg/kg/day).

CONCLUSION

Despite the differences in assumptions, initial conditions, and PBPK model construction, our exposure assessments come very close to those published by Schreiber (1992). Consequently, the excess cancer risk estimates calculated from our model are similar to estimates presented by Schreiber (1992). It seems that the exposure assessment by PBPK models may serve as a valuable tool in the overall risk assessment process for nursing infants.

Based on the PCE exposure assumptions for the mother and nursing child, the EPA cancer risk methodology for PCE, and a human pharmacokinetic lactation model, the following conclusions are presented.

Mother's milk can be contaminated with PCE as a result of occupational exposure to PCE. The amount of PCE ingested by the infant is small, producing theoretical infant cancer risks of 2×10^{-5} to 1×10^{-4} (Table 3.5-2). For nonoccupational PCE exposure, in apartment buildings located above dry cleaning operations, the amount of PCE inhaled and ingested by nursing infants can be substantially more than from an occupational exposure, producing theoretical infant cancer risks ranging from 2×10^{-3} to 1×10^{-2}.

REFERENCES

American Conference of Governmental Industrial Hygienists. 1992. Threshold limit values for chemical and physical agents and biological exposure indices. Cincinnati, OH.

Bagnell, P.C. and H.A. Ellenberger. 1977. Obstructive jaundice due to a chlorinated hydrocarbon in breast milk. *Can. Med. Assoc. J.* 117:1047-1048.

Bolanowska, W. and J. Golacka. 1972. Inhalation and excretion of tetrachloroethylene in men in experimental conditions. *Medycyna Pracy* 23:109-1.

Byczkowski, J.Z. and J.W. Fisher. 1994. Lactational transfer of tetrachloroethylene in rats. *Risk Analysis* 14(3):339-349.

Byczkowski, J.Z., E.R. Kinkead, R.J. Greene, L.A. Bankston, and J.W. Fisher. 1993. Physiologically-based modeling of the lactational transfer of tetrachloroethylene. *Toxicologist* 13:354.

Fernandez, J., E. Guberan, and J. Caperos. 1976. Experimental human exposures to tetrachloroethylene vapor and elimination in breath after inhalation. *Am. Ind. Hyg. Assoc. J.* 37:143-150.

Fisher, J.W., J.M. Gearhart, R.J. Greene, L.A. Bankston, C. Bryant, and S.J. Fortunato. 1993. Estimating the lactation transfer of volatile chemicals in women using a physiological model. *Toxicologist* 13:356.

Greenberg, M.M. and J.C. Parker. 1983. Health assessment document for tetrachloroethylene (perchloroethylene). EPA/600/8-82/005F, July 1985. Government Reports Announcements & Index, Issue 05-1983.

International Commission on Radiological Protection. 1984. No. 23 Report on reference man. W.S. Snyder, M.J. Cook, L.R. Karhausen, G.P. Howells, and I.H. Tipton, eds. Elmsford, NY: Pergamon Press.

New York State Department of Health. 1991. Investigation of indoor air contamination in residences above dry cleaners. Albany, NY: Bureau of Toxic Substance Assessment.

Ramsey, J.C. and M.E. Andersen. 1984. A physiologically based description of the inhalation pharmacokinetics of styrene in rats and humans. *Toxicol. Appl. Pharmacol.* 73:159-175.

Schreiber, J.S. 1992. Summary of an exposure and risk assessment regarding the presence of tetrachloroethylene in human breast milk. *J. Exposure Anal. Environ. Epidem.* 2, Suppl. 2:15-26.

Shelley, M.L., M.E. Andersen, and J.W. Fisher. 1988. An inhalation distribution model for the lactating mother and nursing child. *Toxicol. Lett.* 43:23-29.

Stewart, R.D. 1969. Acute tetrachloroethylene intoxication. *J. Amer. Med. Assoc.* 208:1490-1492.

Stewart, R.D., E.D. Baretta, H.C. Dodd, and T. Torkelson. 1970. Experimental human exposure to tetrachloroethylene. *Arch. Environ. Health* 20:224-229.

Stewart, R.D., D.S. Erley, A.W. Schaffer, and H.H. Gay. 1961a. Accidental vapor exposure to anesthetic concentrations of a solvent containing tetrachloroethylene. *Ind. Med. Surg.* 30:327-330.

Stewart, R.D., H. Gay, D. Erley, C. Hake, and A. Schaffer. 1961b. Human exposure to tetrachloroethylene vapor. *Arch. Environ. Health* 2:516-522.

Ward, R.C., C.C. Travis, D.M. Hetrick, M.E. Andersen, and M.L. Gargas. 1988. Pharmacokinetics of tetrachloroethylene. *Toxicol. Appl. Pharmacol.* 93:108-117.

3.6 GENOTOXICITY OF 1,3,3-TRINITROAZETIDINE

D.E. Dodd

ABSTRACT

1,3,3-Trinitroazetidine (TNAZ) is a highly energetic castable explosive that is being considered by the Department of Defense for military and space applications. Toxicity information is needed to evaluate this compound for potential use as an explosive replacement. A structurally related compound, 1-nitroazetidine, can be converted to 1-nitrosoazetidine, a rodent carcinogen. Genetic toxicity studies with TNAZ were initiated on the possibility that it also might convert or metabolize to a nitroso derivative. The following assays capable of detecting mutations at the gene level were employed: a bacterial (Ames *Salmonella*) test, an *in vitro* forward mutation assay at the hypoxanthine-guanine phosphoribosyl-transferase locus in Chinese hamster ovary cells, and an *in vivo* cytogenetic (mouse bone marrow erythrocyte micronucleus) test. Confirmatory assays were conducted for the *in vitro* bacterial and mammalian cell test systems. The results of the genetic toxicity studies indicated that TNAZ was negative in the three test systems. Thus, TNAZ was negative for the gene mutations in bacterial and mammalian cells and for chromosomal mutations of mouse bone marrow erythrocytes.

INTRODUCTION

The Air Force Armament Laboratory is investigating new explosive ingredients at their High Explosive Research and Development Facility. One compound that shows promise for replacing octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) (CAS No. 2691-41-0), a highly energetic castable explosive, is 1,3,3-trinitroazetidine (TNAZ) (CAS No. 97645-24-4). HMX is used predominantly as a propellant and also in maximum-performance explosives (Rosenblatt et al., 1991). TNAZ is a white, granular solid; it can be synthesized with 98 to 99% purity; and it is recrystallizable from alcohols. Acute toxicity studies with TNAZ on laboratory animals indicate that it is nontoxic dermally, presents no sensitization potential, causes transient eye irritation, and is moderately toxic orally. The Air Force requested information on the genotoxicity potential of this high explosive candidate. Three mutagenic tests were selected for genotoxicity screening: the Ames *Salmonella* test, the hypoxanthine-guanine phosphoribosyl-transferase (HGPRT) test in Chinese hamster ovary (CHO) cells, and the mouse micronucleus assay.

Background Toxicity Data on TNAZ

Pursuant to the request by the U.S. Army Armament Research, Development, and Engineering Center to perform toxicity studies on TNAZ, the U.S. Army Environmental Hygiene Agency initiated acute toxicity, irritancy, and sensitization tests. Results of these tests follow (Weeks, private communication). Sprague-Dawley rats were administered single oral doses of TNAZ dissolved in dimethylsulfoxide (DMSO). The approximate lethal dose (ALD) was 2,222 mg/kg; the ALD for DMSO was >3,333 mg/kg. No deaths were observed when TNAZ acetone solutions were applied dermally to the occluded, shaved skin of New Zealand White (NZW) rabbits and Albino-Hartley guinea pigs at dosages up to 1,480 and 3,333 mg/kg, respectively. Technical grade TNAZ (50% solution in acetone) did not induce a delayed contact sensitization reaction in guinea pigs following the Buehler sensitization protocol. Primary skin and eye irritancy tests (Draize method) were performed on NZW rabbits. TNAZ was not irritating to the intact skin. Neat TNAZ produced slight injury to the cornea and moderate to severe injury to the conjuctiva. These eye irritation results were resolved within 2 days after exposure. In summary, TNAZ was nontoxic and nonirritating dermally, produced no sensitization potential, was of moderate toxicity orally, and caused transient eye irritation.

Proposed Genotoxicity Studies

A chemical related to TNAZ is 1-nitroazetidine. 1-Nitroazetidine can be converted to 1-nitrosoazetidine, which has been extensively investigated for its carcinogenicity and has been shown to be strongly positive in the rat, mouse, and hamster (Lijinsky et al., 1984). This information leads to the concern that TNAZ also might be converted or metabolized to corresponding nitroso derivatives that are carcinogenic. There are no literature citations on TNAZ to support or refute this proposal. Because the genotoxicity of a compound may reflect its carcinogenicity potential, three common assays were selected to investigate the genotoxicity potential of TNAZ. The assays chosen were the Ames *Salmonella*/microsomal test, the CHO/HGPRT forward mutation test, and the mouse *in vivo* bone marrow micronucleus test. These assays were chosen because of their high sensitivity and reliability.

The Ames assay detects both base pair substitution point mutations and/or frameshift mutations. A high percentage of chemicals that elicit a mutagenic response in the Ames assay are potential animal and human mutagens and carcinogens (McCann and Ames, 1976). Because the *Salmonella* assay has been shown to indicate mechanisms of chemical interaction with DNA and has produced few false positives for noncarcinogens, it has been used as the cornerstone of any battery of genotoxicity testing.

The CHO/HGPRT forward mutation assay has been used to determine the mutagenicity of chemicals in mammalian germ cells. The gene for HGPRT is located on the X-chromosome. In an evaluation of Phase III of the U.S. Environmental Protection Agency's (EPA's) genetic toxicology program (Li et al., 1988), the high sensitivity value of the CHO/HGPRT assay was supported. This assay detected mutagenicity of industrial and environmental chemicals from 25 chemical classes and, of the 43 proven carcinogens tested, 40 were found to be positive.

The mouse bone marrow micronucleus assay is a rapid, *in vivo* cytogenetic assay based on the observation that cells with broken chromosomes or impairments of the spindle apparatus often have disturbances in the distribution of chromatin during cell division. Micronuclei are formed from chromosomes or chromosomal fragments left behind during the anaphase. In this assay, polychromatophilic erythrocytes (PCEs) in the bone marrow are scored for the presence of micronuclei. During maturation from erythroblast to erythrocyte, the nucleus is extruded, whereas micronuclei, if present, remain in the cytoplasm. Detection of the micronuclei in nonnucleated cells is thus facilitated and provides a useful index of clastogenicity or anaphase-lag in erythrocytes (Schmid, 1976).

MATERIALS AND METHODS

The Toxic Hazards Research Unit subcontracted the performance of the three genotoxicity assays with a Good Laboratory Practice certified commercial laboratory. The statement of work for the subcontract requested that the three tests be carried out according to EPA's Health Effects Testing Guidelines (40 CFR, Parts 798.5265, 798.5300, and 798.5395, 1 July 1990 edition). Confirmatory assays were conducted for the *in vitro* bacterial and mammalian cell test systems. A brief presentation of the materials and procedures employed for each assay follows. Details of the materials and methods for each test are given in reports by Paika (In Press a,b,c).

TNAZ Test Substance

TNAZ has a structural formula of $C_3N_4H_4O_6$ and is a white, granular solid with a particle size of approximately 100 μm. The crystal density is 1.84 and the onset exotherm temperature is 200 °C. The test material was supplied by J.W. Mitchell, Jr., Director, Systems Safety, Headquarters Air Force Development Test Center, AFSC, Eglin Air Force Base (AFB), FL. Solubility tests were performed by R.B. Nolan, Chemical Research Officer, Eglin AFB, FL. Results of the solubility tests indicated that TNAZ was nonsoluble in water, slightly (10%) soluble in saline, 100% soluble in DMSO or acetone, and nonsoluble in corn oil.

Salmonella typhimurium Reverse Mutation Assay (Ames Assay)

The *Salmonella typhimurium* strains used in this assay were TA98, TA100, TA1535, TA1537, and TA1538. The preincubation technique was used to enhance the sensitivity of the plate incorporation assay. The TNAZ test substance was dissolved in DMSO and administered *in vitro* directly into the test system. Bacteria were exposed to the test substance both in the presence and absence of a metabolic activation system (S9 microsomal fraction of rat liver homogenate obtained from Aroclor 1254-treated rats). Positive control substances with and without metabolic activation systems for all strains and negative control substances were used. Ten concentrations (10,000 to 0.1 μg/plate) were used in the dose range-finding assay; six concentrations (500 to 0.01 μg/plate) were used in the reverse mutation assay.

Gene Mutation at the HGPRT Locus in Cultured CHO Cells Assay

The CHO-K1 cell line was originally derived from the ovary of a female Chinese hamster (*Cricetalus griseus*) obtained from American Type Culture Collection, Rockville, MD. The TNAZ test substance was dissolved in 0.5% DMSO and administered *in vitro* directly into the test system. The CHO cells were exposed to the test substance both in the presence and absence of a metabolic activation system (S9 microsomal fraction of rat liver homogenate obtained from Aroclor 1254-treated rats). Positive control substances with and without metabolic activation systems and negative control (including solvent control) substances were used. Ten concentrations (5.00 to 0.008 mg/mL) were used in the dose range-finding assay, seven concentrations (0.500 to 0.008 mg/mL) were used in the activated mutagenic test, and seven concentrations (0.250 to 0.004 mg/mL) were used in the nonactivated mutagenic test. Duplicate cultures seeded with 5×10^5 cells/flask were used at each dose level. Postexpression, the cells were harvested and reseeded in selection medium at 2×10^5 cells/100-mm dish. Five dishes were plated per dose level, for a total of 1×10^6 cells. Concurrently, 200 cells/100-mm dish were seeded in complete medium for the Parallel Cloning Efficiency Assay.

Mouse Bone Marrow Erythrocyte Micronucleus Test

Healthy male and female albino Swiss mice (*Mus musculus*) were obtained from Charles River Breeding Laboratories (Wilmington, MA). The animals were 7 to 12 weeks of age at the start of the study. The TNAZ test substance was suspended in corn oil and administered by intraperitoneal injection. Mice received three single doses, 24 h apart. In a dose range-finding study, death was observed in mice administered doses ≥ 100 mg/kg (body weight). Five doses were selected for the definitive study: 40, 20, 10, 5, and 1 mg/kg. Positive (mitomycin C) and negative (corn oil) substances also were administered to naive mice. Mice were sacrificed 24 h after the last dose. Bone marrow slides were prepared from the femur. A

total of 1000 PCEs were scored for the presence of micronuclei. The slides were scored blindly to reduce bias associated with the analysis.

RESULTS

Salmonella typhimurium Reverse Mutation Assay (Ames Assay)

Range-Finding Assay. The range-finding assay was performed with strain TA100, using negative control substance plates, and with and without microsomal activation. Some toxicity was observed, as determined by a reduction in the number of spontaneous revertants, a clearing of the background lawn, and by the degree of survival of treated cultures.

The negative control substance plates gave a reference point from which to compare the data. The negative control substance values fell within two standard deviations of the historical mean value for the laboratory or reference literature.

The mean number of revertants per plate was calculated for each concentration (data not shown). A positive result was not observed for any strain because a significant increase in the number of revertant colonies over its corresponding negative control substance was not observed. Because toxicity was detected, dose levels were chosen to bracket toxic and nontoxic levels.

Reverse Mutation Assay. The positive control substance assays consisted of direct-acting mutagens and mutagens requiring metabolic biotransformation. All positive controls exhibited twice the number of colonies as did the negative control substances, demonstrating that the test system was functional with known mutagens (Tables 3.6-1 and -2). The negative control substance plates, for each strain, gave a reference point to compare the test data. Their values fell within two standard deviations of the historical or literature mean values.

The test substance is not considered mutagenic because the number of revertant colonies associated with the test substance did not represent a twofold increase over the number of revertant colonies associated with the corresponding negative control substance (Tables 3.6-1 and -2). The results are considered valid because the positive control substance yielded a mutagenic response, and the values for the negative control substance fell within the 95% confidence limit of the historical background.

A dose response was not observed for the test article in the reverse mutation assay. The results of the assay were confirmed through an independent confirmatory assay (with fresh samples).

TABLE 3.6-1. AMES REVERSE MUTATION ASSAY WITHOUT MICROSOMAL ACTIVATION (Revertants/Plate[a])

	Controls		Test Article (TNAZ) Dose Levels (μg/plate)					
Strain	Positive[b]	Negative[c]	500	50	5	0.5	0.05	0.01
TA98								
Mean	166.0	33.7	0.0	31.0	32.3	32.3	32.3	31.3
SD	7.9	0.6	0.0	1.0	2.1	1.5	3.2	1.2
TA100								
Mean	329.3	135.7	1.7	129.7	127.0	128.0	127.7	133.7
SD	12.3	7.0	2.1	4.7	6.1	2.6	1.5	5.7
TA1535								
Mean	152.0	23.0	0.0	23.0	23.0	22.7	23.0	23.0
SD	5.2	1.0	0.0	1.0	2.6	1.5	1.0	2.6
TA1537								
Mean	105.3	12.3	0.0	10.7	12.7	9.7	12.3	11.7
SD	1.5	0.6	0.0	0.6	0.6	0.6	1.5	1.5
TA1538								
Mean	124.7	18.0	0.0	17.3	17.3	18.3	18.3	17.0
SD	2.1	1.0	0.0	2.1	1.5	1.5	0.6	2.0

[a] All plates were dosed at 100 μL/plate.
[b] The positive control used was sodium azide for strains TA-100 and TA-1535, 2-nitrofluorene for strain TA-98, and 9-aminoacridine for strain TA-1537.
[c] The negative control used in the assay was dimethylsulfoxide.

TABLE 3.6-2. AMES REVERSE MUTATION ASSAY WITH MICROSOMAL ACTIVATION (Revertants/Plate[a])

	Controls		Test Article (TNAZ) Dose Levels (μg/plate)					
Strain	Positive[b]	Negative[c]	500	50	5	0.5	0.05	0.01
TA98								
Mean	174.3	40.3	0.0	40.3	41.3	42.7	42.3	40.3
SD	2.9	0.6	0.0	1.5	2.1	1.5	2.5	1.5
TA100								
Mean	421.3	181.7	11.7	180.0	174.0	177.3	170.7	171.7
SD	15.5	5.7	2.5	4.6	2.0	2.5	2.1	4.0
TA1535								
Mean	187.3	25.7	0.0	26.7	25.7	26.7	26.3	26.3
SD	3.8	2.5	0.0	0.6	2.1	0.6	1.5	0.6
TA1537								
Mean	123.3	14.3	0.0	13.7	14.3	12.7	13.7	14.0
SD	3.8	2.1	0.0	1.5	1.2	1.2	0.6	1.0
TA1538								
Mean	130.0	19.3	0.0	19.7	19.0	17.3	20.7	21.0
SD	5.3	1.5	0.0	0.6	1.7	3.2	1.2	2.0

[a] All plates were dosed at 100 μL/plate.
[b] The positive control used was 2-aminoanthracene for all strains.
[c] The negative control used in the assay was dimethylsulfoxide.

Gene Mutation at the HGPRT Locus in Cultured CHO Cells Assay

Range-Finding Assay. Of the doses of TNAZ tested, the higher dose concentrations at 5.00, 2.00, 1.00, and 0.500 mg/mL were cytotoxic in the activated system (data not shown). Therefore, 0.500, 0.250, 0.125, 0.062, 0.031, 0.016, 0.008, and 0.004 mg/mL doses were utilized.

Mutagenicity Assay. Both in the presence and absence of microsomal S-9 liver enzyme, the test substance failed to induce significantly large numbers of mutant colonies (Tables 3.6-3 and -4). The effect of the test substance on the expression of mutant colonies was similar to untreated (negative) and solvent controls, whereas both positive controls exhibited an increased induction of mutant colonies.

TABLE 3.6-3. HGPRT/CHO MUTATION WITH TNAZ RESULTS WITH ACTIVATION[a]

	Mutant Scoring		Parallel Cloning Efficiency	
Test	Average Mutant Colonies/Dish	Average Surviving Colonies	Average % Plating Efficiency	Mean Mutant Frequency Per 1×10^6 Survivors
Test Substance (0.500 mg/mL)	0.0	29.0	14.5	0.00
Test Substance (0.250 mg/mL)	0.4	146.0	73.0	2.74
Test Substance (0.125 mg/mL)	0.6	156.0	78.0	3.87
Test Substance (0.062 mg/mL)	0.6	150.0	75.0	4.00
Test Substance (0.032 mg/mL)	1.0	159.0	79.5	6.29
Test Substance (0.016 mg/mL)	0.8	165.0	82.5	4.85
Test Substance (0.008 mg/mL)	0.6	135.0	67.5	4.44
Negative Control	0.4	162.0	81.0	2.47
Solvent Control (0.5% DMSO)	0.6	170.0	85.0	3.53
Positive Control (0.3 μL/mL DMN)	18.6	156.0	78.0	119.23

[a] DMSO= Dimethylsulfoxide
DMN = Dimethylnitrosamine

The mutagenicity assay was repeated as a confirmatory assay. These results indicate that the test substance did not induce increased numbers of mutants and is comparable to that of the negative control substance under the conditions utilized in this test system.

TABLE 3.6-4. HGPRT/CHO MUTATION WITH TNAZ RESULTS WITHOUT ACTIVATION[a]

	Mutant Scoring		Parallel Cloning Efficiency	
Test	Average Mutant Colonies/Dish	Average Surviving Colonies	Average % Plating Efficiency	Mean Mutant Frequency Per 1×10^6 Survivors
Test Substance (0.250 mg/mL)	0.0	0.0	0.0	0.0
Test Substance (0.125 mg/mL)	0.0	0.0	0.0	0.0
Test Substance (0.062 mg/mL)	0.0	0.0	0.0	0.0
Test Substance (0.032 mg/mL)	0.6	124.0	62.0	4.84
Test Substance (0.016 mg/mL)	0.8	162.0	81.0	4.94
Test Substance (0.008 mg/mL)	0.4	158.0	79.0	2.53
Test Substance (0.004 mg/mL)	0.6	146.0	73.0	4.11
Negative Control	0.4	163.0	81.5	2.45
Solvent Control (0.25% DMSO)	0.4	156.0	78.0	2.56
Positive Control (0.03 μL/mL 4-NQ)	14.6	145.0	72.5	100.69

[a] DMSO= Dimethylsulfoxide
4-NQ =4-Nitroquinolin-1-oxide

Mouse Bone Marrow Erythrocyte Micronucleus Test

Range-Finding Assay. Immediately after dosing, death due to the toxicity of the test substance was observed among all of the animals dosed at 500 mg/kg. Death also was observed in three out of six animals dosed at 100 mg/kg immediately after dosing. The remaining three animals at the 100-mg/kg dose exhibited tremors. At 10 mg/kg, signs of tremors were observed for all animals. No signs of toxicity were observed at 1.0 mg/kg.

Final Assay. For the final assay, doses were 40, 20, 10, 5.0, and 1.0 mg/kg. At the 40, 20, and 10 mg/kg dose levels, tremors were observed in all animals immediately after injection. At 5.0 mg/kg, 5 out of 10 animals exhibited tremors. The remaining five animals did not exhibit any signs of toxicity. No signs of toxicity were observed in any animals at the 1.0 mg/kg dose level.

There was a statistically significant increase in the number of micronucleated PCEs in the positive control substance group compared to the negative control substance group (Table 3.6-5). In the negative control substance, the average number of micronucleated PCEs per 1000 PCEs did not exceed five (Table 3.6-5).

TABLE 3.6-5. MOUSE MICRONUCLEUS ASSAY WITH TNAZ ANALYSIS OF MICRONUCLEATED CELLS IN BONE MARROW EXTRACT SMEARS

Test	Number of Animals	Number of Micronucleated Cells/1000 PCEs (Mean ± SD)
Positive Control Substance[a]	10	40.60 ± 4.58
Negative Control Substance[b]	10	3.60 ± 0.70
TNAZ - 40 mg/kg	10	4.00 ± 1.05
TNAZ - 20 mg/kg	10	4.40 ± 0.97
TNAZ - 10 mg/kg	10	4.50 ± 0.85
TNAZ - 5 mg/kg	10	4.30 ± 0.82
TNAZ - 1 mg/kg	10	4.60 ± 0.84

[a] Mitomycin C (0.2 mg/kg in corn oil)
[b] Corn oil (40 mL/kg)

Each test and control group was analyzed separately for male versus female animals utilizing a Student t-test to analyze for possible sex differences. Because no statistical significance was noted in the frequency of micronuclei between males and females, the data were pooled and males and females were analyzed as a combined data set (Table 3.6-5).

The frequency of micronucleated PCEs in each dose group was compared to that of the respective negative control substance using analysis of variance and the Newman-Keuls test for confirmation of pairwise comparisons. All results are considered not significant at $p \geq 0.05$. There was a statistically significant increase

in the number of micronucleated PCEs in the positive control substance group compared to the negative control substance group, at $p \leq 0.05$.

The test substance did not produce a statistically significant dose-related increase in the number of micronucleated PCEs or a statistically significant and reproducible positive response at any one of the Test Substance concentrations.

CONCLUSION

The results of the genetic toxicity studies indicated that TNAZ was negative in all three test systems. Results of the *in vitro* bacterial and mammalian cell gene mutation assays were confirmed with independent confirmatory tests. In the three test systems, the positive control substance gave clearly positive results of mutagenicity, indicating that the test systems were able to detect changes in mutation rates. Thus, TNAZ is not considered to have mutagenic potential based on the experimental conditions described and the results obtained from these three genotoxicity assays.

REFERENCES

Li, A.P., R.S. Gupta, R.H. Heflich, and J.S. Wassom. 1988. A review and analysis of the Chinese hamster ovary/hypoxanthine guanine phosphoribosyl transferase assay to determine the mutagenicity of chemical agents. A Report of Phase III of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat. Res.* 196: 17-36.

Lijinsky, W., R.M. Kovatch, and G.L. Knutsen. 1984. Carcinogenesis by nitrosomorpholines, nitrosooxazolidines and nitrosoazetidine given by gavage to Syrian golden hamsters. *Carcinogenesis* 5: 875-878.

McCann, J. and B.N. Ames. 1976. Detection of carcinogens as mutagens in the *Salmonella*/microsome test. Assay of 300 chemicals: Discussion. *Proc. Natl. Acad. Sci. U.S.A.* 72: 5135-5139.

Paika, I.J. In Press a. Genetic toxicity evaluation of 1,3,3-trinitroazetidine. Volume I of IV: Results of *Salmonella typhimurium* reverse mutation assay (Ames assay). Technical Report. Wright-Patterson Air Force Base, OH: Armstrong Laboratory

Paika, I.J. In Press b. Genetic toxicity evaluation of 1,3,3-trinitroazetidine. Volume II of IV: Results of mouse bone marrow micronucleus test. Technical Report. Wright-Patterson Air Force Base, OH: Armstrong Laboratory

Paika, I.J. In Press c. Genetic toxicity evaluation of 1,3,3-trinitroazetidine. Volume III of IV: Results of gene mutation at the HGPRT locus in cultured Chinese hamster ovary cells. Technical Report. Wright-Patterson Air Force Base, OH: Armstrong Laboratory.

Rosenblatt, D.H., E.P. Burrows, W.R. Mitchell, and D.L. Palmer. 1991. Organic explosives and related compounds. In: O. Hutzinger, ed. *The Handbook of Environmental Chemistry*, Vol. 3, Part G, pp. 195-234. Berlin Heidelberg: Springer-Verlag.

Schmid, W. 1976. The micronucleus test for cytogenetic analysis. In: A. Hollaender, ed. *Chemical Mutagens* Vol. 4A, pp. 31-53. New York, NY: Plenum Press.

Weeks, M.H. 1989. Letter to Commander, USAEHA, regarding toxicity study results with TNAZ, 21 September.

3.7 ACUTE TOXICITY EVALUATION OF AMMONIUM DINITRAMIDE

E.R. Kinkead, S.A. Salins, and R.E. Wolfe

ABSTRACT

The Department of Defense is considering replacing ammonium perchlorate with ammonium dinitramide (ADN). Ammonium dinitramide, a class 1.1 explosive oxidizer, would be used in solid rocket engine propellant mixtures and as a high explosive. The first objective of this study was to provide acute hazard information on ADN to industrial hygienists responsible for the safe handling of this material. Because the most likely form of accidental exposure would be by the dermal or oral routes, acute oral and dermal toxicity tests were performed. Oral gavage of ADN solution greater than 1 g/kg resulted in mortality preceded by convulsions. An oral median lethal dose (LD_{50}) of 823 mg/kg was determined for male Fischer 344 rats. Rats receiving nonlethal doses of ADN showed no treatment-related effects when necropsied following a 14-day observation period. Dermal toxicity in New Zealand White rabbits, performed at the U.S. Environmental Protection Agency's limit dose level of 2 g/kg body weight, resulted in no mortality; no clinical signs or differences in clincial pathology were found following a 14-day posttreatment observation period.

INTRODUCTION

The Department of Defense currently is considering replacing ammonium perchlorate with ammonium dinitramide (ADN). Ammonium dinitramide, a Class 1.1 explosive oxidizer, would be used in solid rocket engine propellent mixtures and as a high explosive. No acute or chronic toxicity information is currently available for ADN; however, field reports from exposed personnel indicate that the compound is readily absorbed by the skin, resulting in numbness of the fingers.

Preliminary toxicology information was required for this compound to determine potential acute toxicity hazards that must be addressed. The most significant exposure route expected would be dermal and possible accidental ingestion. This study addresses these potential routes of exposure.

MATERIALS

Test Compound

The ADN [$NH_4N(NO_2)_2$] was supplied by SRI International, Menlo Park, CA. The test compound, a water-soluble powder, is light sensitive and was maintained in an enclosed cabinet. The test compound is known to be contaminated with 1 to 2% ammonium nitrate (AN).

Animals and Animal Husbandry

Male Fischer 344 (F-344) rats, six weeks of age, were purchased from Charles River Breeding Labs, Raleigh, NC. Male New Zealand White (NZW) rabbits weighing between 2 and 3 kg were purchased from Myrtle's Rabbitry, Inc., Thompsons Station, TN. All animals were identified by tattoo and were subjected to a 2-week acclimation period. Rats were group housed (three per cage) in clear plastic cages with wood-chip bedding (Betta-Chip, Northeastern Products Corp., Warrensburg, NY). The rabbits were housed individually in suspended, wire-bottom, stainless steel cages. Water and feed (Purina Formulab #5008 for rats, Purina Rabbit Chow #5320 for rabbits) were available ad libitum, except for 12 h prior to oral dosing. Animal room temperatures were maintained at 21 to 25 °C and the light/dark cycle was set at 12-h intervals.

METHODS

Oral Toxicity

Five male F-344 rats per dose level were fasted 12 h prior to administration of the oral dose. The rats were individually weighed prior to dosing to determine the proper injection volume. The rats were initially dosed at the U.S. Environmental Protection Agency's limit test dose of 5 g ADN/kg body weight. Following that, geometrically spaced dosed levels were used, which allowed for the calculation of a median lethal dose (LD_{50}) using the moving average method of Weil (1952). Surviving rats were weighed on Days 1, 7, and 14 posttreatment. At necropsy, sections of stomach, small and large intestine, liver, kidneys, and gross lesions were sampled for histopathologic examination.

Dermal Toxicity

The backs and sides of five male NZW rabbits were clipped 24 h prior to dosing. A dose of 2 g/kg was applied to the backs of the rabbits and spread evenly to both sides. The dose was kept in place by applying an eight-ply gauze patch over the test substance. Clear plastic wrap was then applied over the entire midsection and was held in place with Vetrap (3M, St. Paul, MN) and elastoplast tape. The test material remained in contact with the rabbit skin for 24 h, at which time the tape, plastic wrap, and gauze were removed and the residual test material was wiped from the skin. Records were kept of body weights (at time of dosing and on Days 1, 7, and 14 posttreatment), signs of toxicity, and mortality. Gross pathology was performed at the termination of the study (Day 14). Sections of skin (treated and untreated), liver, and kidneys were removed for histopathologic examination. Blood samples were taken via the vena cava at necropsy for serum aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, gamma-glutamyl transferase, and alkaline phosphatase measurements. A complete hematology evaluation also was made.

RESULTS

Oral Toxicity

All rats orally gavaged at 5, 2, and 1 g ADN/kg body weight died within 1 h of dosing (Table 3.7-1). In each case, death was preceded by convulsions. Gross examination of the rats showed dilated blood vessels and some lung congestion. Rats gavaged with 0.5 g ADN/kg body weight displayed mild tremors that persisted for several hours. Surviving rats gained weight similar to the control group during the 14-day posttreatment period. Peroral administration of ADN to fasted male rats produced an LD_{50} value of 823 mg/kg. Histopathological examination of tissues taken at necropsy is incomplete.

TABLE 3.7-1. ACUTE ORAL TOXICITY OF ADN

Dose Level (g/kg)	Mortality Ratio	Time to Death
5.0	5/5	<0.5 h
2.0	5/5	1.0 h
1.0	5/5	1.0 h
0.5	0/5	—
0.0 (control)	0/5	—
	LD_{50} = 823 mg/kg	

Dermal Toxicity

The rabbits were treated with 2 g ADN/kg body weight and were maintained for 14 days posttreatment. No mortality occurred and all rabbits appeared unaffected by treatment. Blood evaluations, measured 14 days following treatment, were all within normal limits. Histopathologic examination of tissues taken at necropsy is incomplete.

DISCUSSION

Because acute toxicity data on ADN was not available, the Material Safety Data Sheet (MSDS) that is distributed with ADN is the MSDS for AN, a similar compound. The rat oral LD_{50} for AN is 4.8 g/kg, a dose that would be considered only slightly toxic. However, when ADN was given by gavage to groups of rats at doses much lower than 4.8 g/kg, death occurred rapidly, preceded by convulsions. An oral LD_{50} of 823 mg/kg was established for this compound, which would place it in the moderately toxic classification of compounds having oral LD_{50}s ranging between 0.5 and 5.0 g/kg (Klaassen and Doull, 1980). Ingestion of this

quantity of ADN (823 g/kg) could be equated to a 70-kg man ingesting approximately 58 g of the compound, a quantity not likely to be ingested accidentally.

The rabbit dermal exposure has determined that a dose of 2 g/kg was not lethal and no persistant blood abnormalities resulted. Bartek et al. (1972) determined that rabbit skin is much more permeable to topically applied compounds than is human skin. Therefore, the possibility of toxic effects by this route in humans in unlikely.

REFERENCES

Bartek, M.J., J.A. LaBudde, and H.I. Maibach. 1972. Skin permeability, *in vivo*: Comparison in rat, rabbit, pig and man. *J. Invest. Dermatol.* 58:114-123.

Klaassen, C.D. and J. Doull. 1980. Evaluation of Safety: Toxicologic evaluation. In: J.D. Doull, C.D. Klaassen, and M.D. Amdur, eds. *Casarett and Doull's Toxicology, The Basic Science of Poisons*, 2nd Edition, p. 12. New York, NY: MacMillan Publishing Co., Inc.

Weil, C.S. 1952. Tables for convenient calculation of median-effective dose (LD_{50} or ED_{50}) and instructions for their use. *Biometrics* 8(3):249-263.

3.8 ANALYSIS OF METABOLITES OF TRICHLOROETHYLENE

W.T. Brashear

INTRODUCTION

Trichloroethylene (TCE) has been widely used as a degreasing and cleaning solvent by the Air Force and has been identified as an environmental contaminant commonly found in groundwater. Exposure to TCE is of concern because it has been found to be a rodent carcinogen. To conduct *in vitro* and *in vivo* metabolism and distribution studies, analytical methods need to be developed to quantitate metabolites of TCE in biological samples.

ANALYSIS OF TCE METABOLITES

A method using gas chromatography (GC) with electron capture detection was developed to analyze the metabolites of TCE. The TCE metabolites of interest are dichloroacetic acid (DCA), trichloroacetic acid (TCA), and trichloroethanol (TCOH). These metabolites need to be analyzed in blood, liver, and microsomes. The carboxylic acid metabolites DCA and TCA are not volatile and must be derivatized in order to be analyzed by GC.

Different types of derivatizing agents were evaluated, and dimethylsulfate was chosen because it worked well in blood, liver, and microsomes. The methyl esters formed through dimethyl sulfate derivatization gave interference-free chromatograms from biological samples.

MATERIALS AND METHODS

Trichloroacetic acid, trifluoroacetic acid, DCA, 2,2,2-trichloroethanol, 2,2-dichloropropionic acid, and dimethylsulfate were obtained from Aldrich Chemical Co. (Milwaukee, WI). Sulfuric acid (H_2SO_4) was obtained from Mallinckrodt, Inc. (St. Louis, MO). Sodium dodecyl sulfate, lead acetate, sodium fluoride, and sodium acetate were obtained from Fisher Scientific (Fair Lawn, NJ). Isoniazid was obtained from Sigma Chemical Co. (St. Louis, MO). Chromatographic analysis was performed using a Hewlett-Packard 5890 Series II GC equipped with a Hewlett-Packard 7663A liquid autosampler (Hewlett-Packard, Avondale, PA). Chromatographic separation was performed on a Supelcowax 10 capillary column (30 m$\times$0.32 mm) supplied by Supelco, Inc. (Bellefonte, PA). A Tekmar homogenizer was used to prepare tissue homogenates (Tekmar, Cincinnati, OH). A Haake-Buchler vortex evaporator (Saddlebrook, NJ) was used for heating and vortexing derivatization mixtures.

DERIVATIZATION PROCEDURE

Biological samples containing DCA and TCA were derivatized to form volatile methyl esters. Trichloroethanol is sufficiently volatile to be analyzed by GC without derivatization. The derivatization method was a modification of the procedure used by Maiorino (1980). A 0.1-mL sample of blood, liver, or microsomes was placed into a 2-mL vial with 0.1 mL of water. A 0.1-mL aliquot of 10 μg/mL 2,2-dichloropropionic acid internal standard was added. Samples were placed on ice, and 0.5 mL of concentrated H_2SO_4 was added. After allowing the samples to cool for 30 min, 0.1 mL of dimethylsulfate was added. The samples were placed on a vortex evaporator for 20 min at 60 °C. The samples were allowed to cool, and 1 mL of hexane was added. The DCA and TCA methyl esters were extracted by vortexing for 1 h at 55 °C. Following extraction, the hexane layer was transferred to a 2-mL autosampler vial and was analyzed by GC. The chromatography conditions are summarized in Table 3.8-1.

TABLE 3.8-1. GAS CHROMATOGRAPHY CONDITIONS

Column:	Supelcowax 10 (25 m × 0.32 mm)
Carrier Gas:	95% Argon/5% Methane
Injection Port:	175 °C
Detector:	300 °C
Initial Temperature	70 °C
Initial Time:	15 min
Final Temperature:	190 °C
Heating Rate:	15 °C/min
Final Time:	5 min

A chromatogram of DCA, TCA, and TCOH is shown in Figure 3.8-1. The retention times of DCA, TCA, and TCOH are 8.3, 9.8, and 20.8 min, respectively. The internal standard DPA had a retention time of 5.1 min. The method had a limit of quantitation of 0.5 μg/mL, and a limit of detection of 0.1 μg/mL.

The precision of the method was investigated by including a response check in sample sets, which were analyzed for DCA, TCA, and TCOH. The response check was a sample of control matrix spiked with a known concentration of DCA, TCA, and TCOH. Sample sets contained 5 and 10 μg/mL response checks. The analysis of these response checks provide information on the precision of the method and measured the ability of the standard curve to yield the expected values for DCA, TCA, and TCOH. A summary of the precision data is shown in Table 3.8-2.

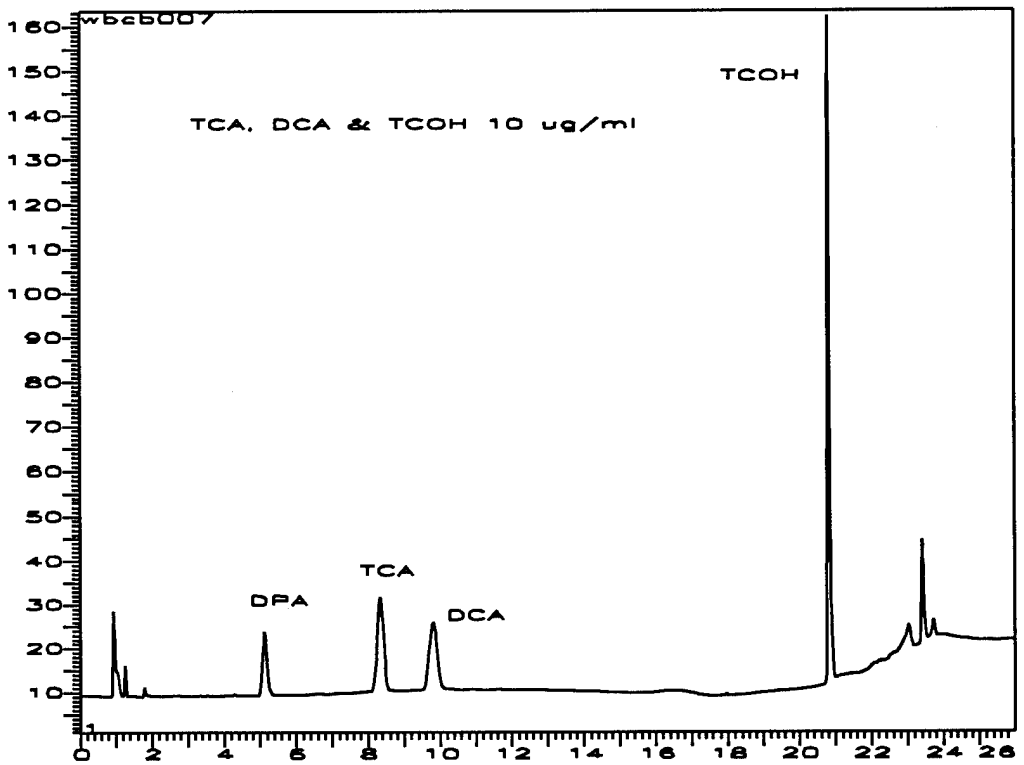


Figure 3.8-1. Chromatogram of DCA, TCA, and TCOH with Internal Standard DPA. The X-axis is retention time in minutes, and the Y-axis is detector response in millivolts.

TABLE 3.8-2. PRECISION OF DCA, TCA, TCOH METHOD

	5 μg/mL			10 μg/mL		
	DCA	**TCA**	**TCOH**	**DCA**	**TCA**	**TCOH**
Liver Slice Samples Spiked at 5 and 10 μg/mL						
Mean	4.9	5.2	4.3	9.8	10.6	8.9
Std Dev	0.6	0.7	0.8	0.6	0.8	1.1
n	19	19	19	55	55	29
CV[a]	13%	12%	18%	6%	7%	13%
Microsomes Spiked at 5 and 10 μg/mL						
Mean	5.0	4.9	4.8	9.8	10.2	9.5
Std Dev	0.3	0.3	0.6	0.7	0.9	1.2
n	33	33	30	34	34	34
CV[a]	7%	6%	13%	7%	9%	12%

[a] CV = coefficient of variation, expressed as percent (Std Dev)/Mean.

The precision of the DCA, TCA, TCOH method was investigated by analyzing calibration checks. The purpose of the calibration checks was to compare analyses of DCA, TCA, and TCOH run on different days. The analysis of calibration checks measures identical samples using different standard curves. Control samples of liver slice homogenate, microsome and cytosol, and blood were spiked with 5 μg/mL DCA, TCA, and TCOH. These spiked control samples were divided into several dozen individual aliquots and were frozen at -20 °C for subsequent analysis. When a sample set was analyzed, a calibration check was included in the analysis. The accuracy data are shown in Table 3.8-3.

TABLE 3.8-3. ACCURACY OF DCA, TCA, TCOH METHOD

	5 μg/mL		
	DCA	**TCA**	**TCOH**
Liver Slice Samples			
Mean	5.6	5.5	5.6
Std Dev	1.0	0.7	1.7
n	13	13	13
CV[a]	18%	12%	31%
Microsomes and Cytosol			
Mean	5.0	5.1	4.7
Std Dev	0.7	0.6	0.8
n	16	16	16
CV[a]	13%	11%	16%

[a]CV = Coefficient of variation, expressed as percent (Std Dev)/Mean.

INVESTIGATION OF EX VIVO METABOLISM OF DCA

Initial efforts to analyze DCA from crude liver homogenates gave results that were not reproducible. Liver samples which were spiked with TCA and TCOH gave linear standard curves and good response checks. However, DCA could not always be detected after spiking with authentic standards of DCA. The stability of DCA in crude liver homogenate was questioned. Investigation of the stability of DCA in fresh crude Fischer 344 (F-344) rat liver homogenates indicated that DCA concentrations decreased. Fischer 344 rat liver homogenates were spiked with varying concentrations of DCA and were allowed to incubate at room temperature for 30 min. Following incubation, the liver homogenates were analyzed for DCA. The results, shown in Figure 3.8-2, show that DCA is lost from these F-344 rat liver homogenates.

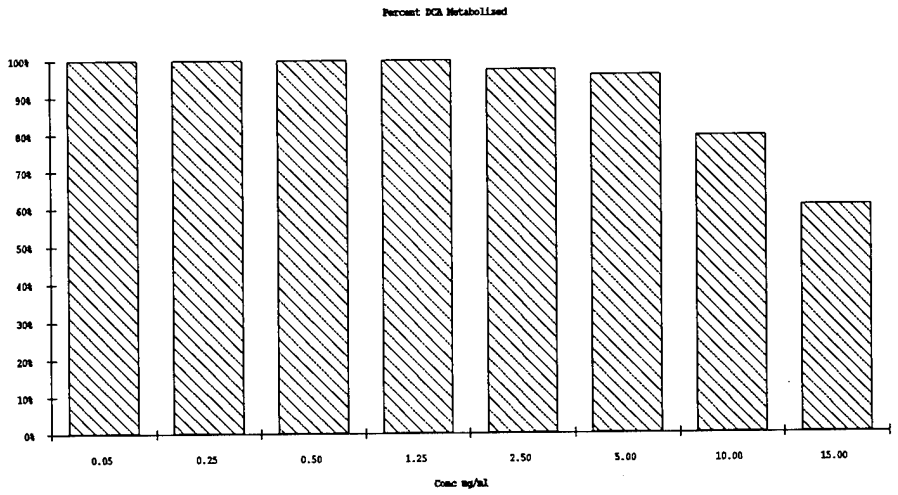


Figure 3.8-2. Percent of DCA Metabolized from Crude Liver Homogenate. The homogenates were spiked with different levels of DCA.

The loss of DCA from liver homogenates could be inhibited by pretreating the liver homogenate with 10% H_2SO_4 or 20% lead acetate for 15 min before adding DCA. This suggests that DCA may be metabolized. One possible route of metabolism could be reductive dehalogenation by cytochrome P-450. This was investigated by pretreating F-344 rat liver homogenates with isoniazid, SKF 525a, and carbon monoxide. These cytochrome P_{450} inhibitors should prevent the metabolism of DCA if the metabolism of DCA is mediated by cytochrome P_{450}. Crude F-344 rat liver homogenates were incubated with the inhibitors for 15 min, DCA then was added to give a final concentration of 5 μg/mL. The results of this experiment, shown in Figure 3.8-3, suggest that the metabolism of DCA is not dependent on cytochrome P_{450} activity. None of the cytochrome P_{450} inhibitors prevented the loss of DCA. Only pretreatment of the liver homogenate with 10% H_2SO_4 inhibited the metabolism of DCA.

Other inhibitors and competitive substrates were investigated. These were added to the F-344 rat liver preparations prior to the addition of DCA. The results of the experiments are summarized in the following Table 3.8-3. DCA metabolism was prevented by 10% H_2SO_4, 20% lead acetate, and sodium dodecylsulfate. The loss of DCA was not inhibited by fluoride. Competitive substrates such as acetate, trichloroacetate, and trifluoroacetate did not prevent the loss of DCA.

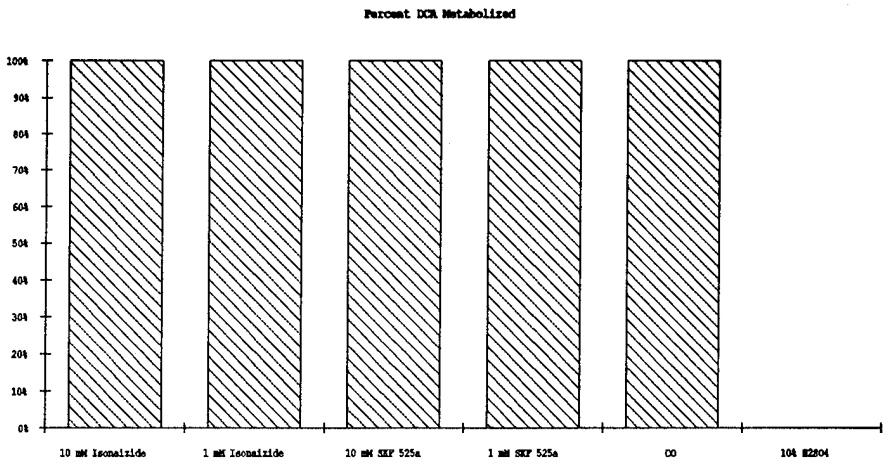


Figure 3-8-3. The Effect of Different Cytochrome P_{450} Inhibitors on DCA Metabolism.

TABLE 3.8-3. THE EFFECT OF CHEMICAL PRETREATMENTS ON DCA METABOLISM

Test Chemical		Metabolize DCA	
		Yes	No
10% Sulfuric Acid	Liver Homogenate		✔
20% Lead Acetate	Liver Homogenate		✔
Sodium Acetate	Liver Homogenate	✔	
Sodium Fluoride	Liver Homogenate	✔	
Isoniazid	Liver Homogenate	✔	
SKF 525a	Liver Homogenate	✔	
Carbon Monoxide	Liver Homogenate	✔	
Trifluoroacetic Acid	Liver Homogenate	✔	
Sodium Dodecyl Sulfate	Liver S9 Fraction[a]		✔
TCA	Liver S9 Fraction[a]	✔	

[a]Liver S9 Fraction is the supernatant from a crude liver homogenate centrifuged at $9000 \times g$.

DISCUSSION

The method developed for the analysis of the metabolites of TCE works well for DCA, TCA, and TCOH. Samples of blood, liver, and microsomes can be analyzed for DCA, TCA, and TCOH and quantitated at levels of 0.5 μg/mL.

Initially, the goal was to develop a single method that analyzes biological samples for DCA, TCA, and TCOH. This method meets that goal, but the precision and accuracy data for TCOH is not as good as for DCA and TCA. This difference could be attributed to the fact that TCOH is more polar than the methyl esters of DCA and TCA. The extraction efficiency into hexane may be lower for the TCOH. It is possible to use a different final extraction solvent such as ethyl acetate or a mixture of hexane and ethyl acetate. Another alternative would be to develop an independent method for TCOH. Headspace analysis by GC is one possibility.

Experiments done while developing this method have shown that *ex vivo* metabolism of DCA occurs in the liver homogenate of the F-344 rat. Metabolic studies by Lin et al. (1993) have shown that in male F-344 rats, glyoxylic acid, glycolic acid, and oxalic acid are the primary metabolites of DCA. These metabolites are shown in Figure 3.8-4. This study used carbon-14 radiolabeled DCA, and high performance liquid chromatography (HPLC) retention times to identify the metabolites. The identity of the metabolites was not confirmed by mass spectrometry (MS). Reviews of the pharmacokinetics of DCA (Stacpoole, 1989) assume that the metabolism of DCA begins with cytochrome P_{450} reductive dehalogenation. Our data suggest that this may not be the case because cytochrome P_{450} inhibitors did not suppress DCA metabolism.

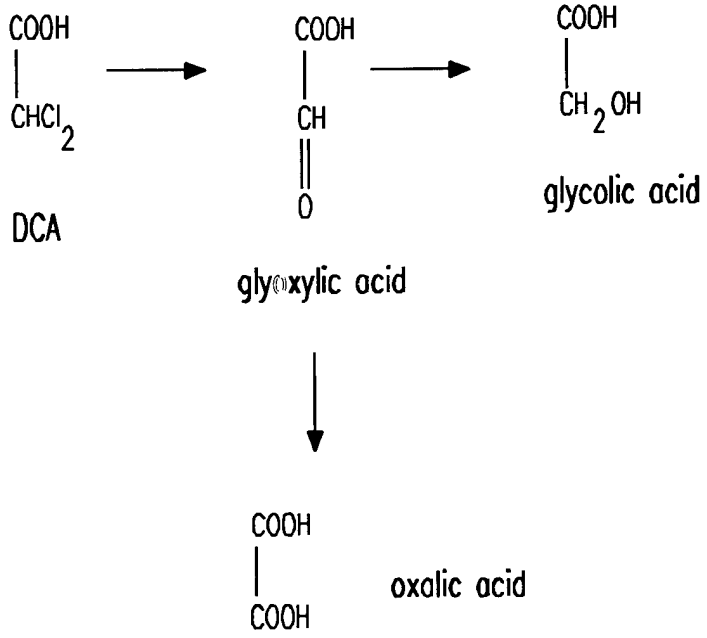


Figure 3.8-4. DCA Metabolism.

This finding brings up questions for future research. One area is to characterize the metabolites of DCA and to identify the responsible enzymes. *In vitro* metabolism studies could be conducted and analyzed by liquid chromatography/MS to confirm glyoxylic acid, glycolic acid, and oxalic acid as metabolites of DCA. This will require the development of an HPLC method to analyze liver samples for DCA metabolites. Once an HPLC method has been established, DCA loss experiments would be conducted to determine whether the loss of DCA can be accounted for in terms of glyoxylic, glycolic, and oxalic acid production.

Investigation of the enzyme(s) responsible for metabolizing DCA is important because cytochrome P_{450} may not be involved. Preparations of microsomes, mitochondria, and cytosol could be tested for metabolic activity towards DCA. Other types of fractionation could be investigated. One possibility would be to use ultrafiltration membranes with different molecular weight cutoffs. These fractions would be tested for metabolic activity towards DCA. When a specific fraction has been found to have activity, it may be possible to isolate the enzyme responsible for the first step in DCA metabolism by affinity chromatography.

REFERENCES

Maiorino, R.M., A.J. Gandolfi, and I.G. Sipes. 1980. Gas-chromatographic method for the halothane metabolites, trifluoroacetic acid and bromide, in biological fluids. *Anal. Toxicol.* 4:250-254.

Lin, E.L., J.K. Mattox, and F.B. Daniel. 1993. Tissue distribution, excretion, and urinary metabolites of dichloroacetic acid in male Fischer 344 rat. *J. Toxicol. Environ. Health* 38:19-32.

Stacpoole, P.W. 1989. The pharmacology of dichloroacetate. *Metabolism* 38(11):1124-1444.

3.9 ACUTE TOXICITY EVALUATION OF HALON REPLACEMENT CF_3I

E.R. Kinkead, S.A. Salins, R.E. Wolfe, H.F. Leahy, and J.H. English[1]

ABSTRACT

Trifluoroiodomethane (CF_3I) is being considered as a replacement compound for Halon 1301. The material would be used as a flooding agent for inflight aircraft and electronic equipment fires and for fire extinguishment in confined spaces. Acute 4-h nose-only inhalation exposures were performed on Fisher 344 rats at 1.0, 0.5, and 0.0% (air only) CF_3I. Specific end points related to thyroid function were determined. Select tissues were examined histopathologically. No signs of toxic stress were noted during exposure or during the 14-day postexposure observation period. Inhalation of CF_3I at concentrations of 1.0 or 0.5% in the atmosphere did not result in any observable toxicological effects.

INTRODUCTION

Environmental concern over the depletion of stratospheric ozone and global warming has led to an international treaty called the Montreal Protocol (1987), which calls for the phase out of Halons by the Year 2000. Presently, the Air Force is using Halon 1301 as a flooding agent for extinguishing inflight aircraft and electronic equipment fires and for fire extinguishment in confined spaces. Because it is believed to have less ozone-depleting activity, trifluoroiodomethane (CF_3I) is being considered as a possible replacement for Halon 1301.

Because CF_3I is not a commercial product, it has not been subject to regulatory compliance studies and very little is known concerning its toxicological properties. However, based on available data from similar compounds such as trifluorobromomethane (Halon 1301), the inhalation median lethal concentration should be quite high (greater than 10%). Because the compound has an iodine atom, it is possible that exposure might interfere with thyroid function (Thomas and Bell, 1982). To determine if inhalation of CF_3I causes changes in the thyroid or thyroid function, inhalation exposures were performed in rats, in which the thyroid hormone (thyroxine [T_4]) was monitored during the postexposure period. Two concentrations were tested with subsequent clinical chemistry evaluations performed immediately following exposure 3 and 14 days postexposure.

---

[1]Army Medical Research Unit, Toxicology Division, Wright-Patterson AFB, OH.

As a research compound, CF_3I is scarce and relatively expensive. To reduce the amount of test compound required to perform these toxicologic evaluations, a nose-only chamber was employed. Chamber volume and air flow are greatly reduced using this apparatus when compared to a whole-body exposure system, resulting in the use of smaller quantities of the test material.

This study was intended to evaluate the potential toxicity of CF_3I following sacute, high-concentration inhalation exposures in rats. It was also designed to determine if changes in thyroid function occurred following the inhalation exposure regimen.

MATERIALS AND METHODS

Test Compound

The CF_3I (CAS 2314-97-8) used in this study was purchased from PCR, Inc., Gainesville, FL. The compound has a formula weight of 195.91 and a boiling point of -22.5 °C. The compound is a liquid and is stored in a cylinder under pressure.

Test Animals

Ninety male Fischer 344 (F-344) rats were purchased from Charles River Breeding Laboratories, Raleigh, NC. The rats were 6 weeks of age upon arrival and 8 weeks of age at time of exposure. All rats were identified by tail tattoo and were subjected to a 2-week quarantine period. Water and feed (Purina Formulab #5008) were available ad libitum except during exposure. Animal room temperatures were maintained at 21 to 25 °C, and the light/dark cycle was set at 12-h intervals. The animals were group housed (two per cage, except during exposure) in clear plastic cages with wood-chip bedding (Betta-Chip, Northeastern Products Corp., Warrensburg, NY). The animals used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals*, prepared by the U.S. Department of Health and Human Services (1985).

Generation and Analysis of Exposure Atmospheres

The CF_3I vapor was generated from a cylinder partially submersed in a water bath maintained at 22 °C. The vapor concentration was controlled using a calibrated Matheson rotometer (Matheson Gas Products, Secaucus, NJ) to measure the volume of gas delivered to a known volume-dilution air input stream. The chamber atmosphere was analyzed using a Varian 3400 gas chromatograph (GC) (Varian Associates, Palo Alto, CA) equipped with a loop injection system with a flame ionization detector. A 15 m×0.53 mm wide bore, fused silica capillary column coated with SPB-5 was used. The column temperature was isothermal at

50 °C. The analysis samples were taken from a centrally located port. The GC sampling system was designed to inject once per minute and cycle every 5 min for integration of five peak areas. This method provided for near continuous analysis of the exposure chamber atmosphere.

The nose-only chamber is a stainless-steel flow-past chamber as described by Cannon et al. (1983). The chamber has 52 ports; 30 were randomly selected for rat exposure. Plexiglas rat restrainer tubes that extended radially outward were plugged into the ports.

Exposure Regimen

Each nose-only exposure duration was 4 h. The exposure groups of 30 rats each were divided into two interim sacrifice groups and one final sacrifice group of 10 rats each. Interim sacrifices occurred immediately following exposure and at 3 days postexposure. A final sacrifice occurred at 14 days postexposure. A total of 30 F-344 rats were included in each of three target exposures of 1.0% (v/v), 0.5%, and 0.0% (air control).

TOXICITY ASSESSMENTS

Records were maintained of body weights, signs of toxicity, and mortality. Euthanasia was via halothane inhalation overdose. At sacrifice, gross pathology was performed on all animals and tissues were harvested for histopathologic examination (Table 3.9-1). Tissues were fixed in 10% neutral buffered formalin and were trimmed and further processed via routine methods for hematoxylin-eosin-stained paraffin-embedded sections (Luna, 1968). Additionally, blood was drawn for select hematology and clinical chemistry assays (Table 3.9-2). Erythrocytes were enumerated on a Coulter counter (Coulter Electronics, Hialeah, FL) and sera for clinical chemistry evaluations were assayed on an Ektachem 700XR (Eastman Kodak, Rochester, NY). Thyroxine and thyroxine-binding globulin (TBG) assays were performed using a DuPont ACA analyzer (DuPont Co., Wilmington, DE).

TABLE 3.9-1. TISSUES HARVESTED FROM CONTROL AND CF_3I-EXPOSED FISCHER 344 RATS FOR HISTOPATHOLOGIC EXAMINATION

Gross Lesions	Liver	Kidneys
Heart	Parathyroid	Adrenals
Lungs	Spleen	Nasal Cavity
Trachea	Thyroid	

TABLE 3.9-2. SERUM CHEMISTRY AND HEMATOLOGY ASSESSMENTS FROM CONTROL AND CF_3I-EXPOSED FISCHER 344 RATS

Albumin	Globulin
Thyroxine-binding globulin	Hematocrit
Thyroxine	Hemoglobin
Alanine aminotransaminase	Red blood cell count
Aspartate aminotransaminase	Total and differential leukocyte count

STATISTICAL ANALYSIS

Comparisons of postexposure BW gains were performed using the repeated multivariate analysis of variance with Scheffe pairwise comparisons (Barcikowski, 1983). A two-factorial analysis of variance with multivariate comparisons was used to analyze the hematology and clinical chemistry data. Histopathology results were analyzed using the Fischer's exact test (Zar, 1974).

RESULTS

Exposure System Analysis

The specified target concentrations of 1.0 and 0.5% CF_3I were maintained during the 4-h exposure period. The exposure mean concentrations were maintained within $\pm 6\%$ of the desired concentrations. Mean$\pm$SD concentrations for each exposure, along with the high and low concentrations, are provided in Table 3.9-3.

TABLE 3.9-3. ANALYSIS OF CF_3I CONCENTRATIONS INHALED BY MALE FISCHER 344 RATS

Target Concentration (%)	1.00	0.50
Mean Concentration (%)	0.99	0.53
Standard Deviation	0.01	0.01
Maximum Concentration (%)	1.01	0.56
Minimum Concentration (%)	0.93	0.52

INHALATION TOXICITY

There were no deaths resulting from exposure. No treatment-related signs of toxic stress were noted during exposure or during the 14-day observation period. Comparison of the mean body weights (MBWs) of

the control and high-exposure groups indicated an initial loss in the CF_3I group at 24 h followed by normal gains thereafter (Table 3.9-4). A similar loss in MBW was noted in the low-exposure group at 24 h.

TABLE 3.9-4. MEAN[a] BODY WEIGHTS OF MALE FISCHER 344 RATS EXPOSED TO CF_3I VIA NOSE-ONLY INHALATION

	Day (Pre- and Postexposure)			
	0	1	7	14
High Exposure	182.1 ± 2.2	178.7 ± 2.8	209.7 ± 2.4	228.4 ± 3.3
Low Exposure	187.0 ± 1.8	180.2 ± 2.0	196.8 ± 3.4	210.1 ± 3.4
Control	183.3 ± 2.1	182.5 ± 2.0	203.2 ± 3.0	222.3 ± 3.4

[a] Mean ± SEM, N=20 at Day 0, N=20 at Day 1, N=10 at Days 7 and 14.

The results of the hematology and clinical chemistry analyses are listed in Tables 3.9-5 and 3.9-6. Several blood parameters were found to be statistically different from control values. The mean TBG and T_4 values of the high-exposure group were statistically significantly different from the control values at 14 days postexposure. Also, the mean TBG value of the low-exposure group was different from the control group immediately following exposure. Tissues examined microscopically showed no lesions of clinical or pathological significance.

DISCUSSION

Acute inhalation of CF_3I at concentrations of 1.0 and 0.5% did not result in any signs of toxic stress during or following exposure. The most important clinical effect of increased iodide concentration in the thyroid is an inhibition of the release of thyroid hormone (Goodman and Gilman, 1985). The difference noted in thyroid hormone levels of the test animals, although in some cases lower than their respective control value, were not significantly lower than the range of control values measured for the respective hormones (TBG range 33 to 39 μg/mL; T_4 range 2.4 to 3.6 μg/mL). Because inhibition of hormone levels in test rats was not found, the statistically significant differences notes in this study are not considered to be of biological significance.

The lack of mortality in rats exposed to 10,000 ppm (1%) CF_3I would indicate that the compound could be classified as "practically non-toxic" (Deickmann and Gerarde, 1969). However, because of its extremely high vapor pressure, a greater hazard might be that of alterations (e.g., anesthesia or ataxia) or asphyxiation if large quantities of this material were to be spilled in a confined area.

TABLE 3.9-5. MEAN[a] HEMATOLOGIC AND CLINICAL CHEMISTRY VALUES OF MALE RATS SACRIFICED POSTEXPOSURE FOLLOWING A 4-h NOSE-ONLY EXPOSURE TO CF_3I

	0 h Postexposure		3 Days Postexposure	
Parameter[b]	High (1.0%)	Control	High (1.0%)	Control
RBC ($\times 10^6/\mu L$)	7.4 ± 0.1	7.3 ± 0.2[c]	7.5 ± 0.1	7.9 ± 0.1
WBC ($\times 10^3/\mu L$)	3.7 ± 0.2	3.4 ± 0.2[c]	6.2 ± 0.2	6.2 ± 0.2
HCT (%)	41.7 ± 0.5	41.0 ± 1.1[c]	42.8 ± 0.8	43.7 ± 0.6
HGB (g/dL)	13.3 ± 0.2	13.1 ± 0.3[c]	13.0 ± 0.2[d]	14.1 ± 0.2
Total Protein (g/dL)	5.8 ± <0.1	6.1 ± 0.1	6.2 ± 0.1	6.2 ± <0.1
Albumin (g/dL)	3.1 ± <0.1	3.3 ± <0.1	3.4 ± <0.1	3.4 ± <0.1
Globulin (g/dL)	2.7 ± <0.1	2.8 ± <0.1	2.8 ± <0.1	2.8 ± <0.1
A/G ratio	1.1 ± <0.1[e]	1.2 ± <0.1	1.2 ± <0.1	1.2 ± <0.1
AST (IU/L)	94.9 ± 5.0	104.5 ± 8.1	160.4 ± 16.1[e]	76.0 ± 1.3
ALT (IU/L)	55.7 ± 1.3[e]	66.8 ± 2.3	58.8 ± 1.0	63.1 ± 1.6
TBG	37.7 ± 0.3	36.0 ± 0.5	39.3 ± 0.2	32.9 ± 0.8
T_4	3.6 ± 0.1	2.9 ± 0.3	4.5 ± 0.3	3.6 ± 0.4
MCV (fL)	56.7 ± 0.2	56.2 ± 0.4[c]	56.9 ± 0.3	55.1 ± 0.1
MCH (pg)	18.1 ± <0.1	18.0 ± 0.1[c]	17.3 ± 0.4	17.7 ± 0.1
MCHC (g/dL)	31.9 ± 0.1	32.0 ± 0.1[c]	30.3 ± 0.7[e]	32.2 ± 0.1

	14 Days Postexposure	
	High (1.0%)	Control
RBC ($\times 10^6/\mu L$)	8.2 ± 0.1	8.4 ± 0.1
WBC ($\times 10^3/\mu L$)	5.7 ± 0.2[d]	6.7 ± 0.3
HCT (%)	44.9 ± 0.4	45.2 ± 0.3
HGB (g/dL)	14.5 ± 0.1	15.0 ± 0.1
Total Protein (g/dL)	6.4 ± <0.1[e]	5.2 ± 0.1
Albumin (g/dL)	3.5 ± <0.1[e]	3.3 ± <0.1
Globulin (g/dL)	2.9 ± <0.1	1.9 ± <0.1
A/G ratio	1.2 ± <0.1[e]	1.7 ± <0.1
AST (IU/L)	100.1 ± 4.6	85.8 ± 1.3
ALT (IU/L)	63.3 ± 1.2[e]	54.5 ± 1.4
TBG	34.1 ± 0.7[e]	38.8 ± 0.5
T_4	4.3 ± 0.4[e]	2.4 ± 0.2
MCV (fL)	54.6 ± 0.2	53.5 ± 0.1
MCH (pg)	17.7 ± 0.1	17.8 ± 0.1
MCHC (g/dL)	32.4 ± 0.1	33.2 ± 0.1

[a] Mean ± SEM, N=10.
[b] RBC=red blood cell, WBC=white blood cell, HCT=hematocrit, HGB=hemoglobin, A/G=albumin/globulin, AST=asparate aminotransaminase, ALT=alanine aminotransaminase, TBG=thyroxine binding globulin, T_4=thyroxine, MCV=mean corpuscular volume, MCH=mean corpuscular hemoglobin, and MCHC=mean corpuscular hemoglobin concentration.
[c] N=9.
[d] Significantly different than control at $p < 0.05$.
[e] Significantly different than control at $p < 0.01$.

TABLE 3.9-6. MEAN[a] HEMATOLOGIC AND CLINICAL CHEMISTRY VALUES OF MALE RATS SACRIFICED POSTEXPOSURE FOLLOWING A 4-h NOSE-ONLY EXPOSURE TO CF_3I

	0 h Postexposure		3 Days Postexposure	
Parameter[b]	Low (0.5%)	Control	Low (0.5%)	Control
RBC ($\times 10^6/\mu L$)	7.6 ± 0.1	7.3 ± 0.2[c]	7.6 ± 0.1	7.9 ± 0.1
WBC ($\times 10^3/\mu L$)	3.8 ± 0.2	3.4 ± 0.2[c]	5.6 ± 0.1	6.2 ± 0.2
HCT (%)	42.8 ± 0.8	41.0 ± 1.1[c]	42.3 ± 0.3	43.7 ± 0.6
HGB (g/dL)	13.7 ± 0.2	13.1 ± 0.3[c]	13.6 ± 0.1	14.1 ± 0.2
Total Protein (g/dL)	6.2 ± 0.1	6.1 ± 0.1	6.3 ± 0.1	6.2 ± <0.1
Albumin (g/dL)	3.4 ± 0.1	3.3 ± <0.1	3.5 ± <0.1	3.4 ± <0.1
Globulin (g/dL)	2.8 ± <0.1	2.8 ± <0.1	2.8 ± <0.1	2.8 ± <0.1
A/G ratio	1.2 ± <0.1	1.2 ± <0.1	1.2 ± <0.1	1.2 ± <0.1
AST (IU/L)	112.2 ± 5.1	104.5 ± 8.1	76.0 ± 1.3	76.0 ± 1.3
ALT (IU/L)	71.9 ± 1.4	66.8 ± 2.3	60.0 ± 1.0	63.1 ± 1.6
TBG	32.6 ± 0.8[d]	36.0 ± 0.5	38.1 ± 0.4	32.9 ± 0.8
T_4	3.9 ± 0.3	2.9 ± 0.3	2.4 ± 0.3	3.6 ± 0.4
MCV (fL)	56.2 ± 0.3	56.2 ± 0.4[c]	56.0 ± 0.2	55.1 ± 0.1
MCH (pg)	17.9 ± 0.1	18.0 ± 0.1[c]	18.0 ± 0.1	17.7 ± 0.1
MCHC (g/dL)	31.9 ± 0.2	32.0 ± 0.1[c]	32.1 ± 0.1	32.2 ± 0.1

	14 Days Postexposure	
	Low (0.5%)	Control
RBC ($\times 10^6/\mu L$)	8.3 ± 0.1	8.4 ± 0.1
WBC ($\times 10^3/\mu L$)	6.4 ± 0.3	6.7 ± 0.3
HCT (%)	44.3 ± 0.6	45.2 ± 0.3
HGB (g/dL)	14.9 ± 0.2	15.0 ± 0.1
Total Protein (g/dL)	5.4 ± 0.1	5.2 ± 0.1
Albumin (g/dL)	3.3 ± <0.1	3.3 ± <0.1
Globulin (g/dL)	2.1 ± <0.1[d]	1.9 ± <0.1
A/G ratio	1.6 ± <0.1	1.7 ± <0.1
AST (IU/L)	95.9 ± 3.3	85.8 ± 1.3
ALT (IU/L)	58.0 ± 1.6	54.5 ± 1.4
TBG	40.5 ± 1.0	38.8 ± 0.5
T_4	2.4 ± 0.2	2.4 ± 0.2
MCV (fL)	53.4 ± 0.2	53.5 ± 0.1
MCH (pg)	18.0 ± 0.1	17.8 ± 0.1
MCHC (g/dL)	33.7 ± 0.1	33.2 ± 0.1

[a] Mean ± SEM, N=10.
[b] RBC=red blood cell, WBC=white blood cell, HCT=hematocrit, HGB=hemoglobin, A/G=albumin/globulin, AST=asparate aminotransaminase, ALT=alanine aminotransaminase, TBG=thyroxine binding globulin, T_4=thyroxine, MCV=mean corpuscular volume, MCH=mean corpuscular hemoglobin, and MCHC=mean corpuscular hemoglobin concentration.
[c] N=9.
[d] Significantly different than control at $p < 0.05$.

REFERENCES

Barcikowski, R.S. 1983. *Computer Packages and Research Design.* Vol. 1:BMDP. Lanham, MD: University Press of America.

Cannon, W.C., E.F. Blanton, and K.E. McDonald. 1983. The flow-past chamber: An improved nose-only exposure system for rodents. *Am. Ind. Hyg. Assoc. J.* 44(12):923-933.

Goodman, L.S. and A.G. Gilman. 1985. *The Pharmacological Basis of Therapeutics,* 7th Edition, pp. 1401-1408. New York: MacMillan Publishing Company.

Luna, L.G., ed. 1968. *Manuual of Histologic Staining Methods of the Armed Forces Institute of Pathology,* 3rd ed. New York: McGraw-Hill.

Thomas, J.S. and J.U. Bell. 1982. Endocrine toxicology. In: A. Wallace Hayes, ed. *Principles and Methods of Toxicology,* p.495. New York: Raven Press.

Zar, J.H. 1974. *Biostatistical Analysis,* Chapter 9, pp.105-106. Englewood Cliffs, NJ: Prentice Hall.

3.10 CLINICAL PATHOLOGY, NECROPSY, AND INHALATION OPERATIONAL SUPPORT FOR A RAT TOLUENE INHALATION STUDY

E.R. Kinkead, S.A. Salins, R.J. Godfrey, and H.F. Leahy

ABSTRACT

Toluene is used extensively by the military in jet fuels, gasoline, explosives, and paints. Menstrual disorders and miscarriages have been reported in women exposed to toluene vapor. Reported *in vivo* studies were performed primarily on male rats, and female reproductive hormone effects were not investigated. This study was designed to determine the effect of toluene inhalation on the estrous cycle and the associated endocrine responses in female Fischer 344 rats. As support for this study, the Toxic Hazards Research Unit (THRU) was requested to provide specified vapor concentrations, with analysis, in the THRU chambers. Specified chamber concentrations of 1000, 500, and 100 ppm toluene vapor were maintained during the three week exposure. Chamber mean concentrations were maintained within ±4% of the desired concentrations.

INTRODUCTION

Toluene (also known as methylbenzene) is used extensively in jet fuels, gasoline, explosives, and paints. Toluene vapor in the workplace has been reported to cause abnormal menstrual cycles in exposed personnel (Matsushita et al., 1975; Linbohm et al., 1990; Axelsson and Rylander, 1984, 1989; Taskinen et al., 1989). Little is known about the effects of toluene on the endocrine reproductive system in the human female. The U.S. Air Force personnel need to be informed about any potential adverse effects of toluene on the reproductive system.

In support of a study to determine female endocrine effects following toluene vapor exposure, the Toxic Hazards Research Unit (THRU) was requested to provide specified vapor concentrations, with analysis, in the THRU inhalation chambers. Support in statistical analysis of data, animal identification, weighings and other PATHTOX requirements also was provided.

MATERIALS AND METHODS

Test Agent

The high performance liquid chromatography grade (99.9% purity) toluene (CAS 108-88-3) used in this study was purchased from Fisher Scientific, Fair Lawn, NJ. A sample of lot 881209 was analyzed using a Hewlett-Packard 5890 Series II gas chromatograph (Hewlett-Packard Co., Palo Alto, CA) linked with a

Hewlett-Packard 5971 mass spectrometer for quality control. The total ion current chromatogram contained one peak, which was identified from its mass spectra by library comparison as methylbenzene.

CAS No.	108-88-3
Chemical Abstract Name	Methylbenzene
F.W.	92.14
Empirical Formula	$C_6H_5CH_3$
Boiling Point	110.6±0.1 °C
Lot No.	881209; 933498

Animals

Upon receipt from Charles River Breeding Labs (Raleigh, NC), female Fischer 344 (F-344) rats, approximately 9 weeks of age, were quality tested as described in Kinkead et al. (1991) and found to be in acceptable health. The animals were group housed (three per cage) in clear plastic cages with wood chip bedding (Betta-Chip, Northeastern Products Corporation, Warrensburg, NY). Water and feed (Purina Formulab #5008, St. Louis, MO) were available ad libitum. Ambient temperatures were maintained at 21 to 27 °C and the light/dark cycle was set at 14/10-h intervals (light cycle starting at 0600 h). The animals used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals*, prepared by the U.S. Department of Health and Human Services (1985).

Generation and Analysis of Exposure Atmospheres

Exposure atmospheres were generated by sparging liquid toluene with filtered, compressed air at room temperature from 0.5-L gas washing bottles (Ace Glass Inc., Louisville, KY) with air flows of 6.5, 3.2, and 0.64 L/min for the 1000-, 500-, and 100-ppm chambers, respectively. The high- and mid-concentration gas wash bottles were maintained in water baths at 23±1 °C. The chamber concentrations were continuously monitored by Miran infrared analyzers (Foxboro, S. Norwalk, CN) at the 13.8 μm absorption band. The path lengths and ranges chosen allowed for more than 60% full scale readout on C/P 8683 recorders (Cole-Parmer Instrument Co., Chicago, IL).

Exposure Regimen and Response Assessments

Twenty female F-344 rats were placed in each of four 690-L inhalation chambers and exposed for 4 h/day, 7 days/week, for approximately 3 weeks to either air only or 1000, 500, or 100 ppm toluene vapor. The chambers were operated at a flow rate of 6.6 ± 0.25 cfm resulting in more than 12 volume exchanges

per hour. Daily exposures routinely occurred between 1000 and 1400 h. The Study Director determined estrous cycles daily (between 0630 and 0900 h) via vaginal cytology. Stage of estrus was determined by examining the vaginal wash via light microscopy. The rats were exposed to air alone on the above schedule for two weeks prior to toluene exposure to establish baseline estrous cycles. Body weights were measured prior to exposure and weekly during exposure as well as at study termination. The rats were sacrificed on the first day of diestrus following three weeks of exposure. Because animals were removed over several days, depending on stage of estrus, there were a total of 26 exposure days.

Euthanasia was via halothane inhalation overdose. At sacrifice, gross pathology was performed and tissues were harvested for future histopathologic examination. Wet tissue weights were determined on liver, brain, pituitary, ovaries, uterus and adrenal gland. Sections of liver, brain, ovaries, uterus, vagina, adrenal, and pituitary sampled for histopathologic examination were fixed in 10% neutral buffered formalin and were trimmed and further processed via routine methods for hematoxylin and eosin-stained paraffin-embedded sections (Luna, 1968).

Additionally, blood was drawn at necropsy via the vena cava for alkaline phosphatase (ALKP) and alanine aminotransferase (ALT) assays, as well as numerous serology assays that were performed by the study originators. The ALKP and ALT evaluations were assayed on an Ektachem 700XR (Eastman Kodak, Rochester, NY). Sera were processed according to procedures in the Ektachem operations manual.

Statistical Analysis

Clinical chemistry assays were performed using a one-factorial analysis with Bonferroni multiple comparison (Barcikowski, 1983). Body weights and organ weights were analyzed using a Dunnett's test within the PATHTOX system.

RESULTS

Chamber Analysis

The specified target concentrations of 1000, 500, and 100 ppm toluene vapor were maintained during the 3-week exposure. All chamber mean concentrations were maintained within $\pm$ 4% of the desired concentrations. Mean concentrations for each exposure chamber, along with the high and low mean concentrations, are provided in Table 3.10-1.

TABLE 3.10-1. ANALYSIS OF TOLUENE CONCENTRATIONS INHALED BY FEMALE FISCHER 344 RATS FOR 3 WEEKS

Target concentration, ppm	1000.0	500.0	100.0
Mean concentration, ppm	1001.0	504.0	101.0
Standard error	2.0	1.0	<1.0
Highest daily average, ppm	1016.0	520.0	103.0
Lowest daily average, ppm	966.0	492.0	98.0

Inhalation Toxicity

A total of 80 F-344 rats were included in the 3-week inhalation study. There were no deaths resulting from the exposures. Toluene-exposed rats consistently gained weight at a similar rate as the control group (Table 3.10-2, Figure 3.10-1). At necropsy, significant concentration-related increases ($p<0.01$) in relative liver weights were observed (Table 3.10-3). A significant increase in absolute and relative ovary weights was noted in all toluene-exposed rats when compared with control animals.

TABLE 3.10-2. MEAN[a] BODY WEIGHTS OF FEMALE FISCHER 344 RATS PRE- AND DURING EXPOSURE TO TOLUENE VIA INHALATION

	Day (Pre- and During Exposure)					
	-14	-7	0	7	14	22
High (1000 ppm)	153.0 ± 5.7	159.9 ± 6.5	164.9 ± 6.4	171.3 ± 5.7	175.7 ± 7.3	180.7 ± 6.6
Medium (500 ppm)	150.1 ± 9.5	158.6 ± 9.6	164.8 ± 8.9	171.7 ± 10.0	177.2 ± 9.1	181.8 ± 10.7
Low (100 ppm)	148.7 ± 5.7	157.2 ± 6.0	163.4 ± 5.9	172.5 ± 6.4	175.7 ± 6.1	180.3 ± 7.1
Control	153.4 ± 4.7	161.6 ± 4.9	166.5 ± 4.9	174.4 ± 5.3	178.6 ± 5.0	182.9 ± 4.6

[a] Mean ± SEM, N=20.

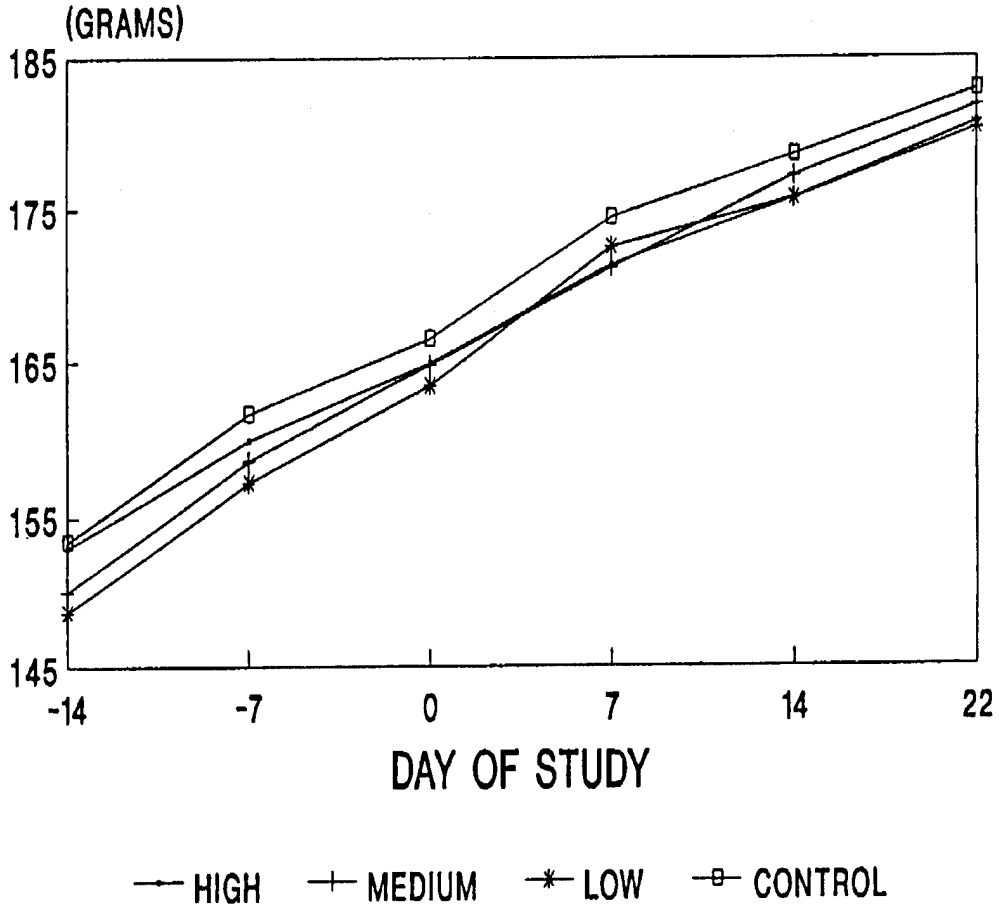


Figure 3.10-1. Mean Weights of Toluene-Exposed Female Fischer 344 Rats.

Analysis of blood chemistry parameters (Table 3.10-4) revealed no significant difference in ALKP between test and control groups. The ALT values were significantly different ($p < 0.05$) at the mid- and low-exposure levels, but not at the high-exposure level. The mean ALT values are within normal limits and, because the difference is not treatment-related, this difference was not considered biologically significant.

Additional results (estrous cycle effects, serum chemistry, body weight effects, and histopathology) will be reported by the government originator.

TABLE 3.10-3. MEAN[a] ABSOLUTE (g) AND RELATIVE (%) ORGAN WEIGHTS OF FEMALE FISCHER 344 RATS SACRIFICED FOLLOWING EXPOSURE TO TOLUENE

	Dose Level			
	1000 ppm	500 ppm	100 ppm	Control
Liver	5.61 ± 0.37[b]	5.65 ± 0.52[c]	5.22 ± 0.58[b]	5.38 ± 0.22[c]
Ratio	3.13 ± 0.17[b,d]	3.11 ± 0.17[c,e]	2.92 ± 0.33[b]	2.94 ± 0.14[c,d]
Brain	1.68 ± 0.05[f]	1.69 ± 0.05	1.69 ± 0.03	1.68 ± 0.05
Ratio	0.94 ± 0.04[f]	0.93 ± 0.05	0.94 ± 0.03	0.92 ± 0.03
Pituitary Gland	0.01 ± <0.01[f]	0.01 ± <0.01	0.01 ± <0.01	0.01 ± <0.01[f]
Ratio	0.01 ± <0.01[f]	0.01 ± <0.01	0.01 ± <0.01	0.01 ± <0.01[f]
Ovaries	0.08 ± 0.01[e]	0.08 ± 0.02[e]	0.08 ± 0.02[e]	0.06 ± 0.01
Ratio	0.04 ± 0.01[e]	0.04 ± 0.01[d]	0.04 ± 0.01[d]	0.04 ± 0.01
Uterus	0.29 ± 0.03	0.29 ± 0.04	0.28 ± 0.03	0.29 ± 0.04
Ratio	0.16 ± 0.02	0.16 ± 0.02	0.15 ± 0.02	0.16 ± 0.02
Adrenal Gland	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
Ratio	0.03 ± <0.01	0.03 ± <0.01	0.03 ± <0.01	0.03 ± <0.01
Terminal Body Weights	180.0 ± 6.7	181.7 ± 10.3	180.0 ± 6.6	183.1 ± 4.8

[a]Mean ± SEM, N=20.
[b]N=16.
[c]N=15.
[d]Significantly different than control at $p < 0.01$.
[e]Significantly different than control at $p < 0.05$.
[f]N=19.

TABLE 3.10-4. MEAN[a] BLOOD CHEMISTRY VALUES IN FEMALE FISCHER 344 RATS EXPOSED TO TOLUENE VAPOR

	Dose Level			
Parameter[b]	1000 ppm	500 ppm	100 ppm	Control
ALKP (IU/L)	232.0 ± 5.7	238.0 ± 5.8	233.0 ± 8.1[c]	232.0 ± 5.3[d]
ALT (IU/L)	69.6 ± 2.5	82.7 ± 6.0[e]	77.7 ± 4.7[c,e]	62.9 ± 2.9[d]

[a] Mean ± SEM, N=19.
[b] ALKP=alkaline phosphatase, ALT=alanine aminotransferase.
[c] N=15.
[d] N=17.
[e] Significantly different than control at $p < 0.05$.

REFERENCES

Axelsson, G. and R. Rylander. 1984. Exposure to solvents and outcome of pregnancy in university laboratory employees. *Br. J. Ind. Med.* 41:305-312.

Axelsson, G. and R. Rylander. 1989. Outcome of pregnancy in women engaged in laboratory work at a petrochemical plant. *Am. J. Ind. Med.* 16:539-545.

Barcikowski, R.S. 1983. *Computer Packages and Research Design.* Vol. 1:BMDP. Lanham, MD: University Press of America.

Kinkead, E.R., S.K. Bunger, E.C. Kimmel, C.D. Flemming, H.G. Wall, and J.H. Grabau. 1991. Effects of a 13-week chloropentafluorobenzene inhalation exposure of Fischer 344 rats and B6C3F$_1$ mice. *Tox. Ind. Health* 7(4):309-318.

Linbohm, M.L., H. Taskinen, M. Sallmen and K. Hemminki. 1990. Spontaneous abortions among women exposed to organic solvents. *Am. J. Ind. Med.* 17:449-463.

Luna, L.G., ed. 1968. *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*, 3rd ed. New York: McGraw-Hill.

Matsushita, T., Y. Arimatsu, A. Ueda, K. Satoh, and S. Nomura. 1975. Hematological and neuro-muscular response of workers exposed to low concentration of toluene vapor. *Ind. Health* 13:115-121.

Taskinen, H., A. Anttila, M.L. Lindbohm, M. Sallmen, and K. Hemminki. 1989. Spontaneous abortions and congenital malformations among the wives of men occupationally exposed to organic solvents. *Scand. J. Work Environ. Health* 15:345-352.

U.S. Department of Health and Human Services. 1985. *Guide for the Care and Use of Laboratory Animals* (Publication No. 86-23). Bethesda, MD: National Institute of Health.

4.1 QUALITY ASSURANCE PROGRAM FOR DEVELOPMENTAL TOXICITY STUDIES: LIQUID PROPELLANT FORMULATION 1846 (XM46)

M.G. Schneider

The Armstrong Laboratory Comparative Medicine Branch (AL/OEVM) conducted a developmental toxicity testing on the Liquid Propellant Formulation 1846 (XM46) provided by the Army Ballistic Research Laboratory. This study was designed to follow the U.S. Environmental Protection Agency (EPA) testing guidelines in 40 CFR Part 798.4900. The Toxic Hazards Research Unit (THRU) provided Quality Assurance (QA) monitoring to determine compliance of this study with the guidelines cited above and with the requirements of the EPA's Good Laboratory Practice Standards (GLPS) stated in 40 CFR Part 792. A subcontractor, Qualtech, Inc., was retained at the request of the government sponsor (U.S. Army) as an outside auditor to review the GLPS compliance status of this study. The report that resulted from the initial site visit indicated that GLPS compliance was satisfactory. The subcontractor also visited the teratology subcontractor during the fetal examinations, and reported compliance with the EPA GLPS. Qualtech, Inc. will conduct a final outside review of this study after THRU QA has completed the final report data audit.

The pathologic (teratology) evaluations for this study were conducted by a subcontractor, Pathology Associates, Inc. (PAI). Several incidents occurred that have delayed the completion of the final report by AL/OEVM. A miscommunication between the Study Director and the supplier of time-pregnant rats on the date of Gestation Day 0 resulted in the dosing with XM46 beginning a day early. Discussions between the Study Director and the sponsor resulted in delayed delivery of the fetal specimens to the subcontractor. Pathology Associates, Inc. encountered delays in collection of the data from the fetal evaluations, and finally encountered computer problems related to the statistical evaluation of the data necessary to complete its final report. At this time there are ongoing discussions between the THRU, the sponsor, and the subcontractor to determine PAI's reporting requirements.

4.2 RANGE-FINDING STUDY FOR A REPRODUCTION ASSESSMENT OF 1,3,5-TRINITROBENZENE ADMINISTERED IN THE DIET OF SPRAGUE-DAWLEY RATS

E.R. Kinkead, R.E. Wolfe, S.A. Salins, C.D. Flemming, D.J. Caldwell[1], C.R. Miller[1], and J.R. Latendresse

ABSTRACT

The soil and groundwater of several military installations targeted for restoration contain measurable quantities of 1,3,5-trinitrobenzene (TNB). As part of the process to develop environmental and health effects criteria, a single-generation reproduction study was performed. Male and female Sprague-Dawley rats received diet containing approximately 800, 400, or 70 mg TNB/kg diet throughout the study. Mating occurred following 14 days of treatment. Dams and pups were maintained through 21 days postpartum, and male rats were necropsied following 35 treatment days. No mortality occurred in parental animals during the study. A treatment-related decrease in food consumption occurred in both sexes, with a concurrent depression of body weight gain. Mean testes weights of the high- and mid-dose rats were significantly less than control testes weights. Likewise, epididymides of test animals weighed significantly less than those of controls. Histopathologic examination showed testicular degeneration in 100% of the high-dose and 70% of the mid-dose animals. Sperm depletion was evident in the lumen of these same groups. All high-dose dams and one mid-dose dam displayed signs of neurotoxicity, primarily head tilt and loss of equilibrium, during the postpartum time period. Histopathologic examination of brain samples from these animals showed vacuolated neuropil in the olivary, deep cerebellar, and vestibular nuclei. Mating indices were normal; however, 4- and 21-day pup survival rates were significantly less in the high-dose group of animals.

INTRODUCTION

Several Army installations targeted for restoration have measurable quantities of 1,3,5-trinitrobenzene (TNB) in the soil and groundwater. 1,3,5-Trinitrobenzene is a dimorphic crystalline solid that is easily dissolved in organic solvents. It is used as an explosive and also has had limited use in the vulcanization of rubber. Both TNB and a similar compound, dinitrobenzene (DNB), are used to produce plastics, herbicides, and paints and can enter domestic-drinking reservoirs via domestic effluent.

---

[1]Army Medical Research Detachment, Walter Reed Army Institute of Research, Wright-Patterson AFB, OH.

The acute toxicity of TNB has been reported by Fitzgerald et al. (1992). The acute oral toxicity of TNB suspended in corn oil was reported as 298 and 275 mg/kg, respectively, in male and female rats and >900 and 702 mg/kg, respectively, in male and female mice. No deaths occurred when the neat TNB was in contact with rabbit skin for 24 h (limit test). 1,3,5-Trinitrobenzene was found to be a mild skin sensitizer in guinea pigs but did not cause acute irritation on rabbit skin. When applied as a powder, the treated eyes of all test rabbits received scores of severe for redness, chemosis, and opacity through 96 h posttreatment. 1,3,5-Trinitrobenzene caused irreversible damage to the ocular tissue and is considered to be corrosive.

Munition workers exposed to TNB have developed skin irritation, liver damage, and anemia (Hathaway, 1977; Morton et al., 1976; Stewart et al., 1945). Animal studies have shown that oral treatment with structurally similar 1,3-DNB or 2,4,6-trinitrotoluene induces anemia and increases methemoglobin concentration. Those compounds also induce liver and spleen hypertrophy and induce degeneration of the germinal epithelial lining of the seminiferous tubules, resulting in decreased spermatogenesis (Cody et al, 1981; Levine et al., 1983, 1984; Furedi et al., 1984a,b). Toxicity studies of TNB following inhalation exposure were not available. Information was not available concerning developmental or reproductive toxicity induced by TNB. Also, no cancer bioassays or epidemiology studies have been reported.

Exposure to TNB can occur through contact with wastewater effluents released from facilities that synthesize, produce, or demilitarize munitions, or from the disposal of solid 2,4,6-trinitrobenzene wastes (Ryon et al., 1984; U.S. Environmental Protection Agency, 1989). Limited information is available on the quantity of TNB in the environment.

PURPOSE

The objective of this range-finding study was to determine if pups could tolerate TNB-treated diet when they began self-feeding during the weaning and postweaning periods. Data from this range-finding study will be used to determine the dose levels to be used in a 90-day modified Screening Information Data Set (SIDS) protocol.

TEST MATERIAL

Test Agent and Doses

The TNB was supplied by the government originator to a separate subcontractor that was responsible for diet preparation. Pertinent chemical and physical properties are listed below.

1,3,5-Trinitrobenzene (TNB)	
Synonym:	Benzenite
CAS #:	99-35-4
Empirical formula:	$C_6H_3N_3O_6$
Formula weight:	231.11
Vapor pressure:	3.2×10^{-6} mmHg at 20 °C

Because of the explosive nature of this compound, the government originator will assume the responsibility of retaining an archive sample.

The test material was mixed in ground Purina Formulab #5002, certified rodent diet meal.

The TNB was administered by the oral route, mixed appropriately in the diet. The mean (N=5) concentrations and (range) per target dose of five prepared diet batches per concentration were 793 (741–837), 403 (367–424), and 66.7 (63.7–69.0) g TNB/kg diet. The TNB-diet preparation and analyses are summarized in Appendix A.

STUDY DESIGN

A description of the study design and evaluations performed in this study were provided in the 1992 Toxic Hazards Research Unit Annual Report (Kinkead et al., 1993).

RESULTS

General Toxicity

No mortality occurred in parental rats during the course of the study. A statistically significant decrease of mean body weight gain of both male and female rats in the high- and mid-dose groups occurred following the first week of treatment and continued through termination of the study. Food consumption was decreased in the same groups of rats through 6 days, after which food consumption returned to pretreatment levels. Food consumption of female rats increased significantly during the postpartum (lactation) period

compared to the premating and gestation periods. However, food consumption in the high-dose females was significantly ($p < 0.01$) less than the other groups at most time points measured.

Food consumption decreased in both sexes of rats when test compound was introduced. Food consumption returned to normal in 4 days in the mid-dose groups and after 6 days in the high-dose groups. For the duration of the study, male rats consumed approximately 30 g diet/day. This consumption rate resulted in the male rats receiving approximately 51, 23, and 3 mg TNB/kg/day in the high-, mid-, and low-dose groups, respectively. The female rats consumed approximately 20 g diet/day during the premating period. This increased to approximately 26 g diet/day during the gestation period and was as high as 70 g diet/day during the lactation period. These data convert to approximately 60, 30, and 4 mg TNB/kg/day through gestation and 110, 55, and 8 mg TNB/kg/day during the lactation period for the high-, mid-, and low-dose groups, respectively.

Rats receiving mid- and low-dose diet displayed circling behavior wherein they would grasp the base of their tail with their teeth and then circle. All high-dose female rats and one mid-dose female rat displayed clinical signs of toxicity during the lactation phase of the study, when food consumption and resultant TNB dose were increased. Clinical signs in these rats began with head tilt, followed by loss of equilibrium, progressing to a "cork-screw-like" motion when moving in the cage. Animals did not recover from these signs through termination of the study, although three of the high-dose rats were returned to control diet for the final 2 weeks of the study. No signs of toxic stress were noted in male rats during the 35-day period.

The TNB-treated rats that received activity tests showed no differences from control animals at any of the interim testing periods. However, high-dose female rats showed a significant increase ($p < 0.05$) in distance traveled and time ambulatory prior to study termination. Other parameters tested (time resting, time stereotypic, number of small movements, clockwise rotations, counter-clockwise rotations, and vertical movements) were not different from control values. There were no differences between TNB-treated and control groups.

The high-dose male rats, sacrificed following mating, showed adverse effects for all measurements of sperm function (Table 4.2-1). The number and concentration of motile cells were greatly reduced in the high-dose group, with no cells traveling in circular patterns. The percent of sperm cells traveling in a circular pattern was reduced at the mid-dose group. Mean absolute testes weight of the high- and mid-dose rats was significantly ($p < 0.01$) less than control testes weight (Table 4.2-2). Likewise, mean absolute weight of epididymides of these two groups were significantly ($p < 0.05$) less than the control group. Relative testes weights were significantly different ($p < 0.01$) in the high-dose group only.

TABLE 4.2-1. SPERM EVALUATIONS FROM RATS ADMINISTERED TNB IN DIET

	Dose			
Parameter	**Control**	**Low**	**Mid**	**High**
Number of Motile Cells	280	249	102	3[a]
Concentration Motile	0.94	0.83	0.32	0.04
Number of Cells Traveling in a Circular Pattern	52	41	1[a]	0[a]
Percent Cells Traveling in a Circular Pattern	19	16	7[a]	0[a]
Percent in Circular Pattern Compared to Total Cells	12	10	0.4[a]	0[a]

[a] Different from control at $p < 0.05$.

TABLE 4.2-2. ABSOLUTE AND RELATIVE ORGAN WEIGHTS[a] OF MALE RATS TREATED WITH TNB

	Dose			
Parameter	**Control**	**Low**	**Medium**	**High**
Testes	3.60 ± 0.11	3.61 ± 0.06	2.91 ± 0.25[b]	1.47 ± 0.06[b]
Ratio (%)	0.68 ± 0.02	0.68 ± 0.01	0.59 ± 0.05	0.32 ± 0.02[b]
Epididymis	0.30 ± 0.02	0.24 ± 0.02	0.18 ± 0.05[c]	0.18 ± 0.01[c]
Ratio (%)	0.06 ± <0.01	0.04 ± <0.01	0.04 ± <0.01[c]	0.04 ± <0.01
Body Weight	529.6 ± 5.56	528.0 ± 3.50	498.4 ± 9.61[c]	469.5 ± 10.17[b]

[a] Mean±SEM, N=10.
[b] Statistically different from control at $p < 0.01$.
[c] Statistically different from control at $p < 0.05$.

Bilateral testicular atrophy was apparent grossly in all rats treated at the high dose. These lesions were confirmed during the histopathologic examination, where 100% of the high-dose and 70% of the mid-dose testes showed degeneration (Table 4.2-3). Sperm depletion and presence of degenerated germ cells in the lumen of the epididymides at several levels (head, body, tail) strongly correlated with the degenerative changes observed in the seminiferous tubules of the testes.

TABLE 4.2-3 INCIDENCE (%) SUMMARY OF SELECTED MICROSCOPIC LESIONS OF MALE RATS FOLLOWING ADMINISTRATION OF TNB IN DIET

Organ/Lesion	Control	Low	Medium	High
Testes (N)	10	10	10	10
Degeneration	0	0	70[a]	100[a]
(severity)[b]	0.0	0.0	1.5[a]	2.8[a]
Epididymis (N)	10	10	10	10
Germ cells	0	0	80[a]	100[a]
Sperm depletion	0	0	70[a]	100[a]
(severity)[c]	0.0	0.0	0.7[a]	4.1[a]
Brain	2	2	2	2
Encephalitis, brain stem	0	0	0	50
(severity)[b]	0	0	0	1

[a] Statistically different from control at $p < 0.01$.
[b] Mean grades of severity based on 0=normal, 1=minimal, 2=mild, 3=moderate, 4=marked, and 5=severe.
[c] Mean grades of severity based on 2=minimal (absence of sperm in the head and decreased sperm in the body of the epididymis), 3=mild (absence of sperm in the head and body of the epididymis), 4=moderate (absence of sperm in the head and body and decreased sperm density in the tail of the epididymis), and 5=severe (absence of sperm in the epididymis).

Discoloration and enlargement of spleens were common findings in female rats from the high- and mid-dose groups. These observations correlated well with the hemosiderosis and extramedullary hematopoiesis (EMH) in the spleens diagnosed microscopically in these groups of rats (Table 4.2-4). The severity of hemosiderosis and EMH appears to be dose related. Brains from five female rats per group were examined histopathologically because of the clinical signs of neurotoxic deficit during treatment. The brains from each of two male rats per group were also examined microscopically. 1,3,5-Trinitrobenzene-induced encephalitis (Tables 4.2-3 and 4.2-4) was found in five of five, three of five, and one of two of the high-dose females, the mid-dose females, and the high-dose males, respectively. The lesions were often bilateral and were most consistently located in the olivary complex of the medulla oblongata and the cerebral peduncle. Other regions where encephalitis also was detected included the inferior calyculus and the facial nuclear region.

TABLE 4.2-4. INCIDENCE (%) SUMMARY OF SELECTED MICROSCOPIC LESIONS OF FEMALE RATS FOLLOWING ADMINISTRATION OF TNB IN DIET

	Dose			
Organ/Lesion	**Control**	**Low**	**Medium**	**High**
Spleen (N)	8	0	6	8
Hemosiderosis	0	-	100[a]	100[a]
(severity)[b]	0.0	-	3.8[a]	4.3[a]
Congestion	0	-	100[a]	88[a]
(severity)[b]	0.0	-	2.2[a]	2.4[a]
Hematopoiesis	0	-	67[c]	75[c]
(severity)[b]	0.0	-	0.8[c]	1.4[a]
Brain (N)	5	5	5	5
Encephalitis, brain stem	0	0	60[c]	100[c]
(severity)[b]	0.0	0.0	1.4[c]	2.0[a]

[a] Statistically significant from control at $p < 0.01$.
[b] Mean grades of severity based on 0=normal, 1=minimal, 2=mild, 3=moderate, 4=marked, and 5=severe.
[c] Statistically significant from control at $p < 0.05$.

Mating and Fertility

A copulation index of 100% was observed for all control and treated groups (Table 4.2-5). The fertility index was 90% in groups given the mid- or the low-dose diet, but was 100% in the high-dose and control groups. No significant treatment-related differences were noted in length of gestation, sex ratio, or mean number of offspring per litter. A decrease ($p < 0.05$) was apparent in the 4- and 21-day survival indices as well as the lactation index in the high-dose group only. Mean body weights (MBWs) of live pups were significantly decreased in a treatment-related manner throughout the 21-day lactation period. The decrease was noted as early as 4 days in both sexes of pups in the high-dose group ($p < 0.01$) and at Day 21 in the mid-dose male pup group. A difference ($p < 0.05$) was noted at Day 14 in the mid-dose male group and at Day 21 in the mid-dose female group. Ringtail was noted in one litter of the high-dose group, four litters of the mid-dose group, and one litter of the low-dose group. Ringtail was not noted in any litter of the control group animals. No other external or visceral malformations were noted in any pups at necropsy.

TABLE 4.2-5. LITTER DATA FOR RATS TREATED WITH TNB

Parameter	Dose Level			
	Control	Low	Medium	High
No. of mated pairs	10	10	10	10
No. of copulated pairs	10	10	10	10
No. of dams with pups born	10	9	9	10
No. of dams with pups alive	10	9	9	10
Gestation index (%)[a]	100.0	90.0	90.0	100.0
Live birth index (%)[b]	98.8	98.5	98.6	93.8
4-Day survival index (%)	98.9	91.0	96.6	86.1[c]
7-Day survival index (%)	100.0	100.0	100.0	94.9
14-Day survival index (%)	98.7	100.0	98.6	100.0
21-Day survival index (%)	100.0	100.0	100.0	86.7[c]
Lactation Index (%)[d]	98.7	100.0	98.6	82.3[c]

[a] Number of females with live litters / Number of females pregnant

[b] Number of live pups at birth / Total number of pups born

[c] Statistically different from control at $p < 0.05$.

[d] Number of pups surviving 21 days / Total number of live pups at 4 days

SUMMARY

The following effects were noted during and following the study.

High-Dose TNB Animals

Body Weight Effects. Male and female animals' MBWs were significantly ($p < 0.01$) depressed when compared to control animals. Mean body weights were depressed by 11 and 15% when compared to control MBWs in the male and female rats, respectively.

Clinical Signs and Neuropathology. One hundred percent of dams showed clinical signs of altered locomotor activity starting postpartum and continuing through necropsy. Brain lesions were noted in 100% of the dams examined at study termination. Similar brain lesions were noted in one of two male rats examined following 35 days of treatment.

Sperm Morphology. Adverse reproductive effects in sperm motility and concentration were noted.

Organ Weights. Testes ($p<0.01$) and epididymides ($p<0.05$) weights were significantly less than controls. The ratio of these organs to body weights were significantly different from controls. Degeneration of these organs was confirmed during the histopathological examination.

Splenomegaly (females). Discoloration and enlargement of spleens in female rats was noted. The incidence of congestion, hemosiderosis, and EMH was also increased.

Locomotor Tests. Distance traveled and time ambulatory were significantly ($p<0.05$) increased in the Opto-Varimex assay.

Reproductive Indices. Reproductive indices were not different from control. Pup survival indices were significantly affected at 4 and 21 days postpartum.

Pup Weights. Pup MBWs were decreased ($p<0.01$).

Mid-Dose TNB Animals

Body Weight Effects. Male and female animals' MBWs were significantly ($p<0.01$) depressed when compared to control animals.

Clinical Signs and Neuropathology. Only a transitory effect was noted in one female rat (head tilt). Circling was noted in some rats. Brain lesions were noted in 60% of dams examined at study termination.

Splenomegaly (females). Discoloration and enlargement of spleens in female rats was noted. The incidence of congestion, hemosiderosis, and EMH was also increased.

Organ Weights. Testes and epididymides weights were significantly less than controls. Degeneration of these organs was confirmed during the histopathological examination.

Locomotor Tests. No differences from control animals were noted in either behavioral test.

Reproductive Indices. No differences from control animals were noted in reproductive indices or survival indices.

Pup Weights. Male pup weights were significantly different at Days 14 and 21; female pups at Day 21 exhibited weight loss.

Low-Dose TNB Animals

Body Weights Body weight gains of male rats were similar to control, whereas female rats were approximately 7% less than control values; however, the differences were not statistically significant.

Clinical Signs and Neuropathology. Circling was noted in some rats during the treatment period. No neural lesions were noted during histopathologic examination.

DISCUSSION

The majority of the seminiferous tubules had marked (or less) alterations. Because spermatogonia were present in most altered tubules, recovery of sperm production would have been likely if the TNB exposure regimen been terminated at that point in time. Collectively, the splenic lesions strongly suggest a TNB-induced dyscrasia of erythrocytes, consistent with regenerative anemia. The distribution and spongiform pattern of the vacuolated neuropil suggest a loss of large neurons in the olivary, deep cerebellar, and vestibular nuclei. Such effects would likely produce the observed clinical manifestations of neurologic disease. However, the distribution (site specific), nature, and extent of the brain lesion should not be overinterpreted in this range-finding study. Specific distribution and development of the brain lesions induced by TNB would best be studied with a time-course experimental design with perfusion fixation and extensive systematic sampling of the brain.

The purpose of this study was to assist in selecting dose levels for a 90-day modified SIDS reproductive study. The dose levels selected for the 90-day SIDS study were 300, 150, and 30 mg TNB/kg diet, which would result in target doses of 25, 12.5, and 2.5 mg/kg/day, respectively. These treatment groups should provide for a high dose that should induce toxicity (testis and sperm effects in male rats, neurotoxic effects in female rats) but not mortality. The selected mid dose is approximately one-half the range-finding study mid dose and is expected to produce minimal observable toxic effects. The lowest level is not expected to produce toxicity. Hematology and bone marrow assessments will be done on the TNB-treated animals. Additional males will be included in the 90-day SIDS study to determine recovery from any male reproductive effects that may be observed during treatment.

REFERENCES

Cody, T.E., S. Witherup, L. Hastings, K. Stemmer, and R.T. Christian. 1981. 1,3-Dinitrobenzene: Toxic effect *in vivo* and *in vitro*. *J. Toxicol. Environ. Health* 7(5):829-847.

Fitzgerald, C.B., N. DiGiulio, L.S. Desai, and G. Reddy. 1992. Acute toxicological evaluation of 1,3,5-Trinitrobenzene. *Acute Toxicity Data* 1(3):169-170.

Furedi, E.M., B.S. Levine, D.E. Gordon, V.S. Rac, and P.M. Lish. 1984a. Determination of the chronic mammalian toxicologic effects of TNT (Twenty-four month chronic toxicity/carcinogenicity study of trinitrotoluene (TNT) in the Fischer 344 rat). Final Report - Phase III, Vol. 1. IIT Research Institute, Project No. L6116, Study No. 11, Chicago, IL. DAMD17-79-C-9120. AD-A168 754.

Furedi, E.M., B.S. Levine, J.W. Sagartz, V.S. Rac, and P.M. Lish. 1984b. Determination of the chronic mammalian toxicologic effects of TNT (Twenty-four month chronic toxicity/carcinogenicity study of trinitrotoluene (TNT) in the B6C3F$_1$ hybrid mouse). Final Report - Phase IV, Vol. 1. IIT Research Institute, Project No. L6116, Study No. 11, Chicago, IL. DAMD17-79-C-9120. AD-A168 754.

Hathaway, J.A. 1977. Trinitrotoluene: A review of reported dose-related effects providing documentation for a workplace standard. *J. Occup. Med.* 19(5):341-345.

Kinkead, E.R., R.E. Wolfe, and S.A. Salins. 1993. Two-generation reproduction study of 1,-3,-5-trinitrobenzene administered in the diet of Sprague-Dawley rats. In: H.G. Wall, D.E. Dodd, A. Vinegar, and M.G. Schneider, eds. 1992 Toxic Hazards Research Unit Annual Report. AL/OE-TR-1993-0105 and NMRI-93-33. Wright-Patterson Air Force Base, OH: Armstrong Laboratory and Naval Medical Research Institute, Toxicology Detachment.

Levine, B.S., J.H. Rust, J.M. Burns, and P.M. Lish. 1983. Determination of the chronic mammalian toxicological effects of TNT (Twenty-six week subchronic oral toxicity study of trinitrotoluene (TNT) in the beagle dog). Phase II, Final Report, IIT Research Institute, Report No. L6116, Study no. 5, Chicago, IL. DAMD 17-79-C-9120, AD-A157 082.

Levine, B.S., E.M. Furedi, D.E. Gordon, P.M. Lish, and J.J. Barkely. 1984. Subchronic toxicity of trinitrotoluene in Fischer 344 rats. *Toxicology* 32:253-265.

Morton, A.R., M.V. Ranadive, and J.A. Hathaway. 1976. Biological effects of trinitrotoluene form exposure below the threshold limit value. *Am. Ind. Hyg. Assoc. J.* 37(1):56-60.

Ryon, M.G., B.C. Pal, S.S. Talmage, and R.H. Ross. 1984. Database assessment of the health and environmental effects of munition production waste products. Final Report, p. 3403. ORNL-6018. (NTIS DE84-016512). New York, NY: Oak Ridge National Laboratory.

Sokal, R.R. and F.J. Rohlf. 1981. *Biometry*, Second Edition, New York, NY: W.W. Freeman and Company.

Stewart, A., L.T. Witts, G. Higgins, et al. 1945. Some early effects of exposure to trinitrotoluene. *Br. J. Ind. Med.* 2:74-82. (Cited in Hathaway, 1977)

U.S. Environmental Protection Agency. 1989. Health and environmental effects profile for 1,3,5-trinitrobenzene. ECAO-CIN-G071. Cincinnati, OH: Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment.

APPENDIX A. DIET PREPARATION AND ANALYSIS

Diet

1,3,5-Trinitrobenzene powder (CAS 399-35-4) was supplied by the U.S. Army Biomedical Research and Development Laboratory. Analysis by high performance liquid chromatography (HPLC) revealed no detectable impurities. Certified powdered Purina Laboratory Chow 5002 was purchased (Ralston Purina Co., St. Louis, MO) and stored at 4 °C until used. The TNB diets were prepared as needed. First, 1.2 g of TNB was added to 50 g of powdered diet in a mortar and thoroughly ground with a pestle. Afterwards, 200 g of the diet was added and mixed for 15 min, then 550 g was added and mixed for an additional 15 min. Finally, the remaining diet (700 g) was added and mixed for 30 min in a mechanical mixer (Kitchen Aid, St. Joseph, MI) for uniform distribution of TNB in the diet. This was verified by determining the TNB concentration in the diet, taken from each of the 1 kg mixtures, by quantitative analysis done by HPLC. The premixed diet (0.8 g/kg) was further diluted with fresh powdered diet to obtain the desired TNB concentration in the lower dose groups. The diet feeders were changed twice a week.

Diet Analysis

Analyses of the TNB-feed mixtures were carried out on acetone extracts of the mixtures, utilizing a Waters 600E chromatography system (Waters, Milford, MA) equipped with a 490E programmable multiwavelength detector operating at 254 nm. The entire chromatography system was interfaced with a Berthold HPLC computer program, Version 1.65 (Berthold, Nashua, NH). The TNB was eluted from a Zorbax C-8 column (9.4 mm×25 cm) (MAC-DOD Analytical, Chadds Ford, PA) with a water-methanol gradient, at a flow rate of 3 mL/min. Working standards were prepared in Burdick and Jackson HPLC-grade high purity methanol (Baxter, Obetz, OH).

4.3 REPRODUCTIVE TOXICITY SCREEN OF LIQUID PROPELLENT 1846 (XM46) ADMINISTERED IN THE DRINKING WATER OF SPRAGUE-DAWLEY RATS

E.R. Kinkead, R.E. Wolfe, S.A. Salins, C.D. Flemming, C.R. Miller[1], and D.J. Caldwell[1]

ABSTRACT

Liquid propellent formulation 1846 (XM46) is being considered as a replacement for solid propellants, both as part of a regenerative injection gun system and as a working fluid in an electrothermal gun system. The XM46 formulation contains hydroxylammonium nitrate, triethylammonium nitrate, and water. This study was performed to evaluate the reproductive toxicity potential of XM46 by the use of a modified Screening Information Data Set protocol Consumption of XM46-treated drinking water resulted in doses of approximately 135, 65, and 16 mg XM46/kg/day. Splenomegaly was a common finding in the XM46-treated rats; in males following 28 days of treatment and in both sexes following 90 days of treatment. A treatment-related decrease in red blood cells, hemoglobin, and mean corpuscular hemoglobin concentration occurred in both sexes. A treatment-related increase occurred in mean corpuscular volume, mean corpuscular hemoglobin, and methemoglobin concentration. Although all parameters have not been evaluated, it appears that continued consumption of XM46-treated drinking water results in hemolytic amenia.

INTRODUCTION

Liquid propellent 1846 (XM46) is a developmental propellent for the next generation Advanced Field Artillery System. This propellent will be used instead of powder charges for the 155-mm cannon. It will be used as part of the regenerative injection gun system and as a working fluid in an electrothermal gun system. The components of XM46, hydroxylammonium nitrate (HAN) and triethanolammonium nitrate (TEAN), are both strongly reducing and oxidizing agents and can react with many organic and inorganic materials (Jet Propulsion Laboratory, 1989). Impurities are generated by reaction of XM46 with components of the gun systems (e.g., metals), manufacturing intermediates, or materials introduced by improper handling after production. Decomposition leads to the formation of nitric acid and ammonium nitrate, with subsequent destabilization of the propellent mixture (Klein et al., 1991; Hansen et al., 1990).

Individuals working with XM46 have reported burning sensations and lesions within 24 h following exposure. Sensitization has been reported in one worker that handled HAN (Parmer et al., 1991). XM46 is a moderate to severe skin irritant and may cause a burning sensation immediately upon contact. If the material

[1]Army Medical Research Detachment, Walter Reed Army Institute of Research, Wright-Patterson AFB, OH.

is not flushed, erythema will be produced in contaminated areas within several hours, followed by severe dermatitis within 24 h (Weller et al., 1989).

When applied to guinea pig skin, XM46 proved to be a strong skin sensitizer. The sensitization response to XM46 may be associated with hydroxylamine, a potent skin sensitizer (Gross, 1985), and to triethylamine, also reported to be a skin sensitizer (Grosselin et al., 1984). Single- and repeat-dose dermal studies on animals have shown that XM46 and HAN penetrate the skin to produce systemic effects. A 21-day repeated dermal application of XM46 and HAN to rabbit skin produced erythrocyte destruction, Heinz body formation, anemia, spleen enlargement, and dermal necrosis (Asaki et al., 1982). Rinsing the skin within 4 h of exposure effectively reduces the severity of the skin lesions (Witt et al., 1991).

XM46 also is a strong eye irritant, producing iritis, chemosis, and corneal opacity, which lasts for up to 1 week. Washing the eye 30 s after application eliminated the corneal opacity and reduced the duration and severity of the remaining effects. Washing at 10 min postapplication further reduced the symptoms, but signs of ocular irritation were still present (Justus and Korte, 1988).

Inhalation of XM46 vapor is not a hazard because the vapor decomposes to almost entirely water. Inhalation of XM46 aerosol, however, may produce respiratory irritation, blood dyscrasia, and elevated methemoglobin (Snodgrass et al., 1985). Extensive genotoxicity studies have been negative and XM46 is classified as a nonmutagen.

This study was intended to evaluate the potential of XM46 to produce alterations in paternal fertility, maternal pregnancy and lactation, and growth and development of offspring of Sprague-Dawley rats.

TEST COMPOUND

The XM46 formulation is a mixture of HAN (61%), TEAN (19%), and water (20%). The compound is a eutectic salt and does not exist as an aqueous solution. The XM46 formulation is acidic and has a density of 1.42 g/mL at 20 °C.

GROUP ASSIGNMENTS AND DOSE LEVELS

Group	Number of Animals		Dose Level of XM46 (mg/L drinking water)	Target Dose Level of XM46 (mg/kg body wt/day)[a]
	Males	Females		
Control	12	12	0.0	0.0
Low	12	12	200.0	3.0
Middle	12	12	1000.0	15.0
High	12	12	2000.0	30.0

[a] Assumed daily water consumption of 50 mL per 300 g rat.

STUDY DESIGN

The test compound was administered orally in drinking water (ad libitum) throughout the study. The drinking water solutions were prepared every 5 to 7 days as needed. Solutions were made up using Building 838 rodent water, which is treated by a reverse osmosis system. All solutions were prepared on a weight to volume basis. The animals were maintained on treated water for 2 weeks prior to mating. The animals were mated within the appropriate treatment level. Dams and one-half of the male groups were maintained through gestation, weaning, and through a total of 90 days on study.

EXPERIMENTAL EVALUATIONS

Evaluations and assessments performed on parental and F1 rats are similar to those of the 1,3,5-trinitrobenzene study detailed in the 1992 Toxic Hazards Research Unit Annual Report (Kinkead et al., 1993).

RESULTS

General Toxicity

No mortality occurred in parental rats during the course of the study. No treatment-related differences were noted in mean body weights of treated rats when compared to their respective control group. Water consumption was significantly decreased in both sexes of treated rats when the test compound was introduced. The water consumption of the treated rats continued to be significantly less than controls throughout the 90-day study. For the duration of the study, male rats consumed approximately 30, 30, and 35 mL/day resulting in a dose of 135, 65, and 16 mg XM46/kg/day for the high-, mid-, and low-dose groups, respectively. The female rats consumed approximately 18, 22, and 25 mL/day during the premating and postweaning periods, resulting in a dose of 140, 80, and 20 mg XM46/kg/day for the high-, mid-, and low-dose groups, respectively. During gestation, the dose increased to 230, 120, and 30 mg XM46/kg/day, and during lactation, the dose was 375, 220, and 50 mg XM46/kg/day for each group, respectively.

One-half of the male rats (six/group) were necropsied following the mating period (28 days treatment). A treatment-related splenomegaly was evident at this time period. Relative spleen weights were increased by 400, 290, and 140% at the high-, mid-, and low-dose levels, respectively. Following 90 days of treatment, the remaining male rats were sacrificed. Relative spleen weights measured at sacrifice were increased by 550, 290, and 130% at the high-, mid-, and low-dose levels, respectively. Other organs weighed at necropsy were not different from those of the control animals.

Splenomegaly also was evident in the female rats sacrificed after 90 days of treatment. Relative spleen weights were increased by 645, 375, and 128% in the high-, mid-, and low-dose groups, respectively.

Relative kidney weights of the high-dose female rats also were significantly increased over control values. The remaining tissues weighed at necropsy did not differ from those of the control group.

A treatment-related decrease in red blood cells, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration occurred in both sexes of rats at the conclusion of the study. These same parameters also showed a significant decrease in male rats following 28 days of treatment. Methemoglobin concentrations measured at the conclusion of the study were increased in both male and female rats. The methemoglobin concentrations in male rat groups were increased by approximately 475, 425, and 200% over control values in the high-, mid-, and low-dose treatment groups, respectively. One-half of the female rat groups (six/group) received untreated water (no XM46) for 24 h prior to sacrifice while the remaining rats continued on treated water until sacrifice. The female rats receiving untreated water for 24 h had methemoglobin values approximately 275, 280, and 125% above control values, whereas those receiving treated water through sacrifice were increased approximatley 580, 450, and 175% over controls.

The treatment showed no adverse effects on mating because 100% of the animals mated. The fertility index was 90% in groups given the control and high-dose treatment, but was 100% in the mid- and low-dose groups. No significant treatment-related differences were noted in length of gestation, sex ratio, gestation index, or mean number of offspring per litter. During the 21-day lactation phase, mean pup weights showed no statistically significant difference between treated and control groups.

Incomplete Evaluations

Many parameters are still being evaluated. Sperm morphology, behavioral tests, and histopathology assessments are in progress. Results from these evaluations will be included in the final technical report on this study.

REFERENCES

Asaki, A.E., L.W. Metker, J.G. Harvey, and E.A. Haight. 1982. Health hazard evaluation of liquid monopropellants, Phase 3, Range finding studies on the effects of hydroxylammonium nitrate in animals, Special Study No. 75-51-0132-84. Aberdeen Proving Ground, MD: U.S. Army Environmental Hygiene Agency.

Gross, P. 1985. Biological activity of hydroxylamine: A review. *CRC Crit. Rev. Toxicol.* 14(1):87-115.

Grosselin, R.E, R.P. Smith, and H.C. Hodge. 1984. *Clinical Toxicology of Commercial Products*. 5th Edition.

Hansen, R., E. Backoff, H.J. DeGrieff, and N. Klein. 1990. Processes for assessing the thermal stability of HAN-based liquid propellants, Final Report. U.S. Army Ballistics Research Laboratory Contractor Report 635. Aberdeen Proving Ground, MD: U.S. Army Ballistics Research Laboratory.

Jet Propulsion Laboratory. 1989. Safe liquid propellant technology. Report on liquid propellant logistics systems study. Phase II. JPL Report D-6876. U.S. Army Armament Research, Development and Engineering Center.

Justus, J.D. and D.W. Korte. 1988. Ocular irritation evaluation with eye wash regimen of liquid propellant 1846. Institute Report No. 299. Presidio of San Francisco, CA: Letterman Army Institute of Research.

Kinkead, E.R., R.E. Wolfe, and S.A. Salins. 1993. Two-generation reproduction study of 1,3,5-trinitrobenzene administered in the diet of Sprague-Dawley rats. In: H.G. Wall, D.E. Dodd, A. Vinegar, and M.G. Schneider, eds. *1992 Toxic Hazards Research Unit Annual Report*, pp. 220-230. AL/OE-TR-1993-0105, NMRI-93-33. Wright-Patterson Air Force Base, OH: Armstrong Laboratory and Naval Medical Research Institute.

Klein, N., C.S. Leveritt, and P.G. Baer. 1991. The effects of composition variation and nitric acid on the stability and reactivity of the HAN-based liquid propellants. U.S. Army Ballistics Research Laboratory Technical Report 3179. Aberdeen Proving Ground, MD: U.S. Army Ballistics Research Laboratory.

Parmer, D.L., W.G. Palmer, D.A. Smart, and R.A. Finch. 1991. Developmental medical research on liquid gun propellants. In: H.J. Clewell III, ed. *Conference on Chemical Risk Assessment in the DOD: Science, Policy, and Practice*, pp. 95-104. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

Snodgrass, H.L., A.E. Asaki, J.G. Harvey, and L.W. Metker. 1985. Health hazard evaluation of liquid monopropellants, Phase 4, Subchronic inhalation of hydroxylammonium nitrate. Special Study No. 75-51-1-0132-85. Aberdeen Proving Ground, MD: U.S. Army Environmental Hygiene Agency.

Weller, R.E., J.E. Morris, B.J. McClanahan, and R.F. Jostes. 1989. Evaluate the dermal toxicity of nitrate compounds and liquid gun propellants. Final Report, Project Number 87PP7806. Frederick, MD: U.S. Army Medical Research and Development Command, Fort Detrick.

Witt, W.M., R.M. Parker, J.D. Thurman, and P.A. Gosnell. 1991. Unpublished results.

5.1 STATISTICAL DESCRIPTION FOR SIX NAVAL POPULATIONS

C.D. Flemming

INTRODUCTION

This project explores the effects of considering specific military subpopulations in estimating the potential for chemical toxicity. Populations of interest may be identified because of differences in their exposure characteristics or in their response to an exposure dose. In some cases, such as chemicals in drinking water, the populations exposed are quite varied. In other cases, exposures occur for specific groups that are actively using a chemical or are close to its use. Civilian or military workers would be an example of the latter case.

Physiological variations also can define populations of interest. These variations may affect either the absorption, distribution, metabolism, or elimination (pharmacokinetics), or the mechanisms of toxicity (pharmacodynamics) of a chemical. Evaluation of differences in pharmacokinetics using physiologically based pharmacokinetic (PBPK) models is the focus of this report.

The general choices were a function of known population samples and characteristics of SEa, Air, Land teams (SEALs), aviators, and fleet sailors. The SEALs were believed to be a trimmer, more athletic community. The aviators were known to have to meet the requirements of periodic, stringent flight physicals. The fleet sailors were chosen to provide a benchmark for comparisons, and they represent most of the active duty personnel.

The results of this project will be used to describe the statistical distribution of each subpopulation and to do Monte Carlo simulations on the subpopulations for the evaluation of differences in pharmacokinetics. Another use of the results will be to aid the Navy in revising its physical fitness standards.

METHODS

Data Collection

The populations and sample sizes used in this report were largely dependent upon the availability of data from the various communities within the Navy. All data were obtained from various Navy research/data holding facilities. Sample sizes were chosen to avoid statistical inadequacy. In selecting the number from each community, there was no intention of having a "proportionate sample" of the communities in the Navy. For mixed sex populations (i.e., aviators and fleet), every effort was made to ensure that the female population was adequately represented.

Outliers

Through descriptive statistics (minimum and maximum), the potential for outliers was evaluated among the Naval populations. Age, body fat, body weight, and body height were correlated; hence, a multivariate method of outlier identification was used. The method was principal component analysis. The method determines factors (axes) that represent the correlational structure of the original variables (Barnett, 1984). The Mahalanobis distances from each case to the centroid (mean of age, body fat, body weight, and body height) for the original data were computed. Also, the Mahalanobis distances from each case to the factor scores were computed. The difference between the these distances was computed. Distances have a chi-squared distribution for large samples. If the chi-square for the difference is significant ($p < 0.01$), the case is considered an outlier. An outlier was eliminated if it was an obvious data entry error.

Estimating the Distribution for Each Population

The determination of a distribution requires measures of the following components: central tendency (mean and median), variation (variance, minimum, maximum, and range), horizontal movement (skewness), vertical movement (kurtosis), correlation among the variables (correlation and covariance), and the theoretical distribution (normal, log-normal, and others). The distributional properties of body fat (%), body weight (kilograms), and body height (centimeters) were calculated for each population. The Wilk-Shapiro ($5 \leq n \leq 2000$) test of normality (Dixon, 1990) or the skewness-kurtosis chi-squared test of normality ($n > 2000$) was used to test the normality of the data or the log of the data.

Modeling the Correlational Structure of Variables

Body fat versus body weight and body weight versus body height graphs were constructed. A mathematical model was fit and validated for each graph. The mathematical model was not limited to one independent variable. The model validation was done using residual analysis.

Equality of Populations

The equality of population means was tested using a one factorial (population) multivariate analysis of variance on body fat, body weight, and body height. The assumption of equality of population variances was tested using a Levene's multivariate test for variances. If the Levene's test was significant ($p < 0.05$), Box-Cox transformations were applied to the data. A one factorial multivariate analysis of variance was applied to the transformed data.

RESULTS

Outliers

Because the data were generated by other laboratories within the Navy, there was a need to identify any recording errors. When the statistical multivariate test of outliers (principal component analysis) was applied to each population, outliers were identified. Table 5.1-1 shows the sample size with the original sample size in parentheses. The percent column indicates the percentages of the sample sizes that were outliers, but the only outliers that were removed were obvious typographical mistakes. The other type of outliers was not removed because there was no scientific reason for removal. The female Naval fleet had one outlier (1.6), which was an obvious typographical mistake because the rest of the data were rounded to the nearest whole number. The aviator data were reduced because the body fat data were missing.

TABLE 5.1-1. OUTLIERS RESULTS FOR EACH POPULATION ($p < 0.01$)

Population	Sample Size	Percent
BUDs	39	3
SEALs	48	4
Male Aviators	150 (192)[a]	7
Female Aviators	38 (58)[a]	11
Male Fleet	2411	8
Female Fleet	317 (318)[b]	8

[a] Original sample size. Sample size was reduced because there were missing data.
[b] Original sample size. Sample size was reduced because there was an outlier that was an entry error.

Determining the Distributions of the Population

The body fat (%) was either normally distributed (Basic Underwater Demolition teams [BUDs], male aviators, female aviators, and male fleet) or log-normally distributed (SEALS and female fleet) (Table 5.1-2). The SEALs showed a different distribution for body fat than did the BUDs, who are trainee SEALs. The SEALs increase their body fat to prepare for a mission because mealtime is unknown. The body weight (kg) was either normally distributed (BUDs, SEALs, male aviators, and female aviators) or log-normally distributed (male fleet and female fleet) (Table 5.1-3). The body height (cm) was normally distributed for all of the military populations (Table 5.1-4).

TABLE 5.1-2. DISTRIBUTION TEST FOR BODY FAT (%)

Population	Distribution	Statistics	p-Value
BUDs	Normal	0.96	0.24
SEALs	Log-normal	0.97	0.40
Male Aviators	Normal	0.98	0.18
Female Aviators	Normal	0.96	0.20
Male Fleet	Normal	2.68 (2)[a]	0.26
Female Fleet	Log-normal	0.98	0.29

[a] Skewness-kurtosis test of normality using a chi-squared distribution (degrees of freedom).

TABLE 5.1-3. DISTRIBUTION TEST FOR BODY WEIGHT (kg)

Population	Distribution	Statistics	p-Value
BUDs	Normal	0.99	1.00
SEALs	Normal	0.96	0.13
Male Aviators	Normal	0.98	0.61
Female Aviators	Normal	0.96	0.12
Male Fleet	Log-normal	1.96 (2)[a]	0.37
Female Fleet	Log-normal	0.99	0.78

[a] Skewness-kurtosis test of normality using a chi-squared distribution (degrees of freedom).

TABLE 5.1-4. DISTRIBUTION TEST FOR BODY HEIGHT (cm)

Population	Distribution	Statistics	p-Value
BUDs	Normal	0.98	0.86
SEALs	Normal	0.98	0.79
Male Aviators	Normal	0.97	0.06
Female Aviators	Normal	0.96	0.18
Male Fleet	Normal	5.90 (2)[a]	0.05
Female Fleet	Normal	2.73 (2)[a]	0.25

[a] Skewness-kurtosis test of normality using a chi-squared distribution (degrees of freedom).

The age ranges for the Naval populations seem to reflect the amount of training and physical fitness required for the population (Table 5.1-5).

TABLE 5.1-5. AGE (years) BASIC STATISTICS

Population	Mean	Median	Std. Dev.	Minimum	Maximum
BUDs	22	21	2.4	19	28
SEALs	26	25	4.5	19	36
Male Aviators	32	30	7.6	21	51
Female Aviators	28	26	6.3	21	45
Male Fleet	30	28	7.3	18	56
Female Fleet	29	28	6.2	18	50

The body fat means (Table 5.1-6) divided into three groups: (1) BUDs; (2) SEALs, male aviators, and male fleet; and (3) female aviators and female fleet. In body fat, the average distance from the mean (standard deviation) differed significantly among the subpopulations.

TABLE 5.1-6. BODY FAT (%) BASIC STATISTICS

Population	Mean	Std. Dev.
BUDs	$10.44^{a,b,c,d,e}$	2.15
SEALs	$14.18^{c,e}$	3.44
Male Aviators	$15^{c,d,e}$	4.47
Female Aviators	22^{d}	6
Male Fleet	16	5.39
Female Fleet	24^{c}	5.57

[a] Significantly different than SEALs at $p < 0.05$.
[b] Significantly different than male aviators at $p < 0.01$.
[c] Significantly different than female aviators at $p < 0.01$.
[d] Significantly different than male fleet at $p < 0.01$.
[e] Significantly different than female fleet at $p < 0.01$.

The body weight means (Table 5.1-7) separated into two groups: (1) BUDs, SEALs, male aviators, and male fleet; and (2) female aviators and female fleet. The body weight variances were not equal among the populations.

TABLE 5.1-7. BODY WEIGHT (kg) BASIC STATISTICS

Population	Mean	Std. Dev.
BUDs	76[a,b]	5.74
SEALs	79[a,b]	7.94
Male Aviators	81[a,b]	9.17
Female Aviators	62[c]	7.87
Male Fleet	81[b]	12.21
Female Fleet	62	8.66

[a] Significantly different than female aviators at $p < 0.01$.
[b] Significantly different than female fleet at $p < 0.01$.
[c] Significantly different than male fleet at $p < 0.01$.

The body height means (Table 5.1-8) separated into two groups: (1) BUDs, SEALs, male aviators, and male fleet; and (2) female aviators and female fleet. The variances for the body height were not equal among the populations.

TABLE 5.1-8. BODY HEIGHT (cm) BASIC STATISTICS

Population	Mean	Std. Dev.
BUDs	177.7[a,b]	6.3
SEALs	177.1[a,b]	5.1
Male Aviators	180[a,b]	6
Female Aviators	166[c]	6.6
Male Fleet	178.2[b]	7.3
Female Fleet	164.9	6.7

[a] Significantly different than female aviators at $p < 0.01$.
[b] Significantly different than female fleet at $p < 0.01$.
[c] Significantly different than male fleet at $p < 0.01$.

Correlational Structure of Variables

For the BUDs, body weight was correlated with body fat and highly correlated with body height (Table 5.1-9). For the SEALs, age was correlated with body fat, and body weight was highly correlated with body fat and body height (Table 5.1-10). For the male aviators, age was highly correlated with body fat, and

body weight was highly correlated with body fat and body height (Table 5.1-11). For the female aviators, body fat was highly correlated with body weight (Table 5.1-12). For the male fleet, body weight was highly correlated with age, body fat, and body height; and body fat was highly correlated with age and was correlated with body height (Table 5.1-13). For the female fleet, body weight was highly correlated with body fat and body height (Table 5.1-14).

TABLE 5.1-9. PEARSON CORRELATION COEFFICIENTS FOR BUDs

	Body Fat	Body Weight	Body Height
Age	0.3120	0.1679	0.0822
Body Fat		0.3194[a]	0.0196
Body Weight			0.6718[b]

[a] Significant at $p < 0.05$.
[b] Significant at $p < 0.01$.

TABLE 5.1-10. PEARSON CORRELATION COEFFICIENTS FOR SEALs

	Body Fat	Body Weight	Body Height
Age	0.2919[a]	0.0926	0.0602
Body Fat		0.6301[b]	0.1034
Body Weight			0.5560[b]

[a] Significant at $p < 0.05$.
[b] Significant at $p < 0.01$.

TABLE 5.1-11. PEARSON CORRELATION COEFFICIENTS FOR MALE AVIATORS

	Body Fat	Body Weight	Body Height
Age	0.3102[a]	0.0589	0.0159
Body Fat		0.5536[a]	0.0990
Body Weight			0.5132[a]

[a] Significant at $p < 0.01$.

TABLE 5.1-12. PEARSON CORRELATION COEFFICIENTS FOR FEMALE AVIATORS

	Body Fat	Body Weight	Body Height
Age	-0.1520	-0.1108	0.0913
Body Fat		0.6992[a]	-0.1955
Body Weight			0.2469

[a] Significant at $p < 0.01$.

TABLE 5.1-13. PEARSON CORRELATION COEFFICIENTS FOR MALE FLEET

	Body Fat	Body Weight	Body Height
Age	0.2934[a]	0.1417[a]	0.0224
Body Fat		0.6642[a]	0.0452[b]
Body Weight			0.5459[a]

[a] Significant at $p < 0.01$.
[b] Significant at $p < 0.05$.

TABLE 5.1-14. PEARSON CORRELATION COEFFICIENTS FOR FEMALE FLEET

	Body Fat	Body Weight	Body Height
Age	0.0519	0.0506	-0.0151
Body Fat		0.6420[a]	-0.0235
Body Weight			0.5451[a]

[a] Significant at $p < 0.01$.

DISCUSSION

Age and body height are generally not used in PBPK models, but the data indicate the need for those variables. No attempt should be maded to incorporate the age and body height in the PBPK models. The Monte Carlo simulation of body fat and body weight, which are in the PBPK models, should be found using the multivariate normal distribution. For the variables of the subpopulations that are log-normal, take the logarithm and apply the multivariate normal distribution to the data.

Because body fat and body weight were correlated with age, the Navy's body fat standards should consider age and body height. When age increased, the body fat had a tendency to increase. Because most of the people in the study were still in the Navy, it would imply that the increase in body fat did not affect the performance of an individual.

The study indicated that the lowest to highest body fat was the trainee male and female Navy personnel. The female Navy personnel had a body weight of approximately 62 kg; whereas the male Navy personnel had a body weight in the high 70 to low 80 kg range. The female personnel body height was about 10 cm lower than that of the male personnel.

REFERENCES

Barnett, V. and T. Lewis. 1984. *Outliers in Statistical Data*, 2nd ed. New York: John Wiley & Sons.

BBN Software Products Corporation. 1988. *RS/1 User's Guide*, ver. 4. Cambridge, MA.

Dixon, W.J., M.B. Brown, L. Engelman, and R.I. Jennrich. 1990. *BMDP Statistical Software Manual,* vol. 1. Berkeley, CA: University of California Press.

SAS Institute Inc. 1990. *SAS Procedures Guide*, ver. 6. Cary, NC.

SAS Institute Inc. 1990. *SAS/STAT User's Guide*, ver. 6. Cary, NC.

5.2 DETERMINATION OF DOSE-RESPONSE EFFECT AND NO OBSERVABLE EFFECT LEVELS IN FISCHER 344 RATS FOLLOWING REPEATED GAVAGE OF TRICRESYLPHOSPHATE AND MIL-H-19457C HYDRAULIC FLUID

E.R. Kinkead, R.E. Wolfe, and S.A. Salins

ABSTRACT

One of the triaryl-based hydraulic fluids presently in use in Naval vessels, MIL-H-19457C, has been found to cause adverse endocrine and reproductive effects following repeated gavage treatments. Quantities of MIL-H-19457C hydraulic fluid aboard Navy ships range from thousands to tens of thousands of gallons. This study was designed to determine the dose–response relationships following gavage treatment with MIL-H-19457C and tricresylphosphate (TCP) on the following endocrine and reproductive indices: estrous cycle, estradiol, sperm count and motility, and morphology of primary and secondary reproductive organs. Repeated daily gavage of TCP and MIL-H-19457C for up to 10 weeks resulted in no mortality and did not influence body weight gains in either sex of rat. Daily vaginal cytology monitorying revealed an extended estrus for the high dose MIL-H-19457C group only. Target organs for both compounds appear to be adrenal and liver. Relative testis weights were increased in the MIL-H-19457C rats following 10 weeks of treatment. No treatment-related effects were noted in any of the groups examined at 5 or 10 weeks posttreatment.

INTRODUCTION

Triaryl phosphates, such as tricresyl phosphate (TCP) and tributylphenyl phosphate (TBP), have many industrial uses as plasticizers and functional fluids, such as oil-based coolants, lubricants, and hydraulic fluids (Toy, 1977). Triaryl phosphate-based hydraulic fluids described by Military Specification H-19457 are used extensively in Navy hydraulic systems, including aircraft and weapons elevators. Because of the fire-resistance of these compounds, as well as their excellent lubricating qualities, there are currently no suitable substitutes for many Navy applications.

Certain triaryl phosphates such as tri-*o*-tolylphosphate are known esterase inhibitors and have been found to cause delayed neurotoxic effects in humans (Doull et al., 1979). Others, such as TBP, do not cause this type of neurotoxicity.

One of the triaryl phosphate-based hydraulic fluids presently in use in Naval vessels, MIL-H-19457C, has been studied extensively in this laboratory. This hydraulic fluid is composed predominantly, but not exclusively, of TBPs. An acute battery of toxicological screens, including acute delayed neurotoxicity evaluation, found MIL-H-19457C hydraulic fluid to have minimal acute toxicity (Gaworski et al., 1986).

Repeated inhalation exposures of rats, hamsters, and rabbits (6 h/day for 21 days) to 250 mg/m^3 resulted in increases in relative liver weights in rats only. Exposure of rats, hamsters, and rabbits to lower concentrations of 100 and 10 mg/m^3 for 90 days had similar effects on the liver as well as increased relative kidney weights in the high-exposure rat group (Wall et al., 1990). Additionally, lesions were noted in the adrenal glands and ovaries of rats following the 90-day inhalation exposure. In the same study, MIL-H-19457B (another triaryl phosphate-based hydraulic fluid) caused testicular degeneration in addition to adrenal and ovarian changes.

Many organophosphate compounds affect reproduction (Dunnick et al., 1984; Harbison et al., 1976; Krause, 1977; Hanna and Kerr, 1981; Oishi et al., 1982). Other studies initiated by the National Toxicology Program have indicated similar endocrinologic changes in rats exposed to TCPs (Hejtmancik et al., 1986). Using a continuous breeding protocol, male and female Swiss mice (CD-1) exposed to TCP were shown to have impaired fertility in both sexes of mice in the F_0 generation and affected sperm motility at the lowest dose (0.005% in diet) in F_1 males (Chapin et al., 1988). Abnormal sperm morphology was observed following repeated doses of 100 mg/kg of TCP in Long-Evans rats (Carlton et al., 1987). Recently, a comparative toxicologic evaluation of MIL-H-19457C and TCP showed abnormal structural and functional reproductive effects caused by both compounds in female Fischer 344 (F-344) rats and germ cell degeneration in male rats gavaged with TCP at 400 mg/kg per day (Latendresse et al., 1992a,b,c, 1993). Endocrine effects were noted in all treatment groups gavaged daily with MIL-H-19457C (1.68, 1.12, and 0.56 g/kg) throughout a 63-day breeding period (Latendresse et al., 1992a,b,c, 1993).

MIL-H-19457C hydraulic fluid was found to be rapidly absorbed following dermal administration (Kinkead et al., 1991). Repeated daily dermal treatment at 1.68 g/kg for up to 28 days determined the ovary, adrenal, kidney, and liver as target organs for this route of exposure, similar to effects determined from the gavage study of Latendresse et al. (1992a,b,c, 1993).

Triaryl phosphates are reproductive toxins. These compounds are used extensively in Navy hydraulic fluids, and Naval personnel will be exposed to these hydraulic fluids. Because these compounds cannot be replaced, it is necessary to know the exposure level that will minimize adverse effects. It is also necessary to know whether the toxic effects will resolve when exposure is withdrawn.

TEST MATERIALS AND METHODS

A detailed description of the methods and experimental evaluations performed for this study was provided in the 1991 Toxic Hazards Research Unit Annual Report (Kinkead et al., 1992). Results from the TCP positive control animals were reported in the 1992 Toxic Hazards Research Unit Annual Report (Kinkead et al., 1993).

RESULTS

MIL-H-19457C Hydraulic Fluid Results

A total of 80 male and 80 female F-344 rats were included in this phase of the study. No deaths occurred in either sex during the posttreatment observation period. Mean body weights of the hydraulic fluid-treated rats are shown in Figure 5.2-1. Gavage treatment with MIL-H-19457C hydraulic fluid did not influence the increase in mean body weights of female rats during the study. However, body weight gains of male rats from the high- and mid-dose groups were significantly less than their respective control group starting at treatment Week 6 and continuing through Week 1 posttreatment.

Daily vaginal cytology monitoring of the hydraulic fluid-treated and control female rats revealed an extended estrus for the high-dose group. Mean ($\pm$ SEM) estrous cycle lengths (days) were 5.66 $\pm$ 0.15, 4.96 $\pm$ 0.04, 4.99 $\pm$ 0.03, and 4.99 $\pm$ 0.02 for the high, mid, low, and control groups, respectively. The cycle length of the high level group was statistically significantly different ($p < 0.01$) from the other treated groups and the control group.

Hematocrit values measured during the course of the study showed no differences between treated and control groups. Analysis of blood chemistry parameters for male and female rats are listed in Tables 5.2-1 and -2. Total protein and albumin values were increased during treatment in the high-dose male rats. Similarly, albumin values were increased in the female rats during the treatment period, but total protein was increased only at 3 weeks of treatment. Plasma acetylcholinesterase values were significantly depressed at the high- and mid-dose levels of both sexes of rats during the daily gavage treatment period. No depression in plasma acetylcholinesterase was noted during the nontreatment observation period (Table 5.2-3).

Treatment-related increases in relative liver and adrenal weights were noted in both sexes of rats following 3 weeks of treatment (Tables 5.2-4 and -5). Increased liver weights were noted in all treatment groups, and increased relative adrenal weights were noted in the high-dose male group and the high- and mid-dose female groups. Increases also occurred in the relative kidney weights of the high- and mid-dose male groups as well as the mid-dose female group. Liver, kidneys, and adrenals continued to be increased in relative weight following 10 weeks of gavage treatment (Tables 5.2-6 and -7). Relative testis weights also showed a treatment-related increase following the 10-week period. All organs of treated groups appeared to have recovered during the posttreatment observation period because no differences in absolute or relative organ weights were noted when compared to the respective control groups (Tables 5.2-8 through -11).

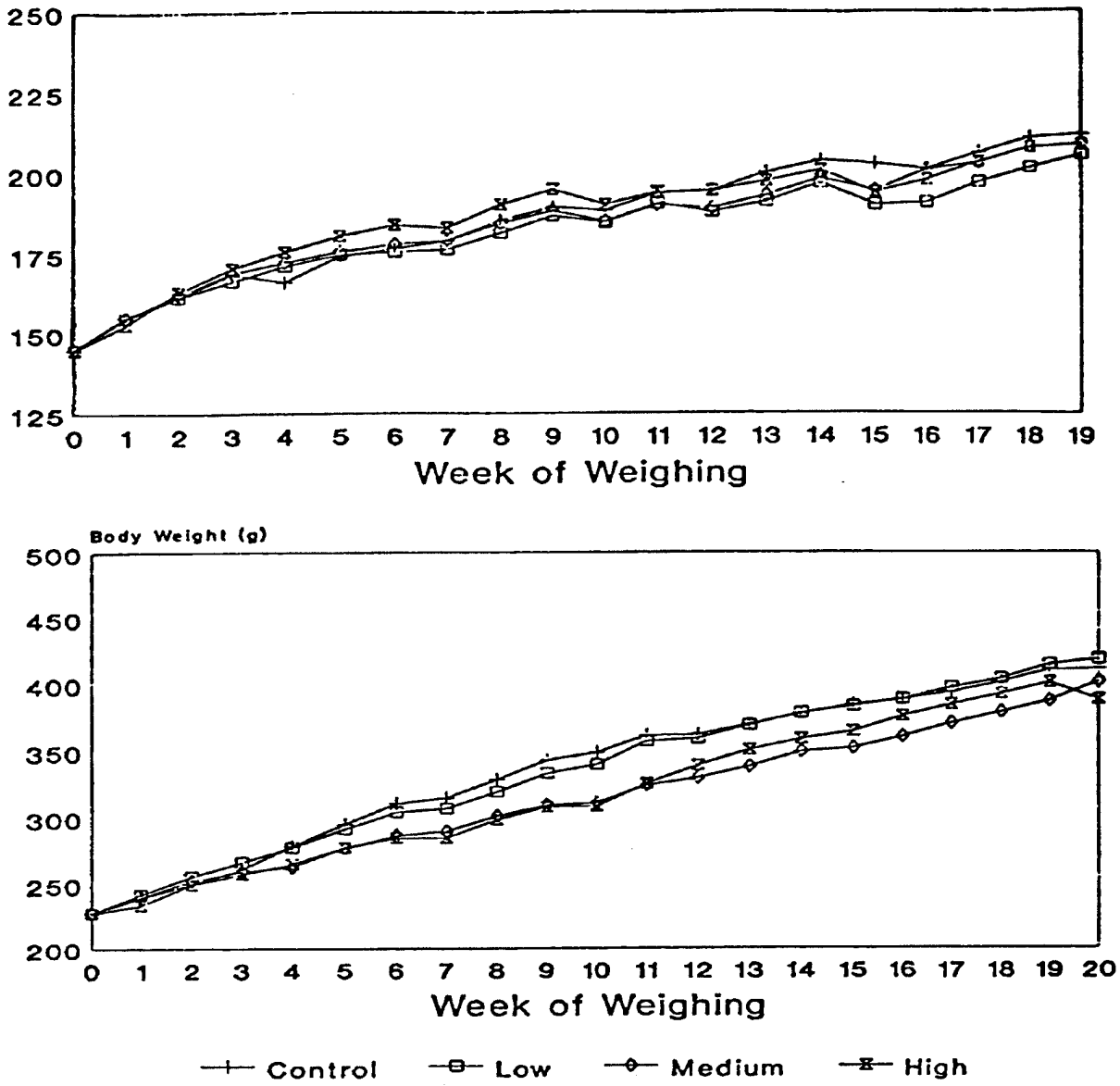


Figure 5.2-1. Mean Body Weights of Female (Top) and Male (Bottom) Fischer 344 Rats Following Repeated Oral Administration of MIL-H-19457C for 10 Weeks. N=20 for Weeks 0 through 3; 15 for Weeks 4 through 10; 10 for Weeks 11 through 19; and 5 for Week 18 through study termination.

TABLE 5.2-1. MEAN[a] SERUM CHEMISTRY PARAMETERS FOR MALE RATS FOLLOWING REPEATED GAVAGE TREATMENT OF MIL-H-19457C

Parameter[b]	Dose			
	Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
3 Weeks				
Total Protein (g/dL)	6.6 ± 0.1	6.8 ± 0.1	7.2 ± 0.1	7.8 ± 0.1[c]
Albumin (g/dL)	4.0 ±<0.1	4.2 ± 0.1	4.5 ± 0.1[c]	5.0 ±<0.1[c]
AST (IU/L)	117.0 ± 8.2	105.0 ± 6.7	107.0 ± 11.3	127.0 ± 8.3
ALT (IU/L)	62.6 ± 5.8	67.2 ± 7.1	78.0 ± 12.4	145.4 ± 16.6[c]
10 Weeks				
Total Protein (g/dL)	6.5 ± 0.1	6.6 ± 0.1	6.9 ± 0.2	7.1 ± 0.1[d]
Albumin (g/dL)	3.7 ±<0.1	3.9 ±<0.1	4.0 ± 0.1	4.2 ±<0.1[c]
AST (IU/L)	134.8 ± 3.9	136.2 ± 10.9	111.6 ± 13.0	145.4 ± 10.7
ALT (IU/L)	65.2 ± 8.8	90.4 ± 17.6	80.6 ± 13.3	136.2 ± 30.0[c]
9 Weeks Posttreatment				
Total Protein (g/dL)	------[e]	------[e]	------[e]	------[e]
Albumin (g/dL)	3.8 ± 0.1	3.9 ±<0.1	3.9 ±<0.1	3.8 ±<0.1
AST (IU/L)	144.2 ± 9.0	133.4 ± 6.9	135.4 ±10.2	144.8 ± 5.8
ALT (IU/L)	102.4 ± 7.1	91.0 ±12.1	98.0 ± 8.5	95.0 ± 7.2
18 Weeks Posttreatment				
Total Protein (g/dL)	6.9 ± 0.1	6.9 ± 0.1	6.7 ± 0.1	6.8 ± 0.1
Albumin (g/dL)	3.7 ±<0.1	3.8 ±<0.1	3.7 ± 0.1	3.7 ±<0.1
AST (IU/L)	184.4 ±18.3	194.4 ±30.1	166.2 ±11.0	169.6 ± 8.2
ALT (IU/L)	110.0 ±26.5	156.6 ±59.4	116.2 ±21.1	118.2 ±19.2

[a]Mean ± SEM, N=5.
[b]AST=aspartate aminotransferase, ALT=alanine aminotransferase.
[c]Different from control at p<0.01.
[d]Different from control at p<0.05.
[e]Parameter not evaluated at this posttreatment period.

TABLE 5.2-2. MEAN[a] SERUM CHEMISTRY PARAMETERS FOR FEMALE RATS FOLLOWING REPEATED GAVAGE TREATMENT OF MIL-H-19457C

Parameter[b]	Dose			
	Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
3 Weeks				
Total Protein (g/dL)	6.1 ± 0.1	6.3 ± 0.1	6.4 ± 0.1	7.1 ± 0.2[c]
Albumin (g/dL)	3.5 ± 0.1	3.7 ± 0.1	3.9 ± 0.1	4.5 ± 0.1[c]
AST (IU/L)	115.0 ± 8.0	106.0 ± 8.0	103.0 ± 9.0	102.0 ± 6.0
ALT (IU/L)	47.6 ± 2.3	51.2 ± 3.9	66.6 ± 13.5	69.2 ± 12.1
10 Weeks				
Total Protein (g/dL)	6.6 ± 0.1	6.7 ± 0.1	6.7 ± 0.1	7.1 ± 0.1
Albumin (g/dL)	3.9 ± <0.1	4.0 ± 0.1	4.0 ± 0.1	4.4 ± 0.1[c]
AST (IU/L)	106.0 ± 9.0	91.0 ± 3.0	87.0 ± 4.0	109.0 ± 9.0
ALT (IU/L)	48.8 ± 1.9	47.0 ± 1.1	46.6 ± 2.3	77.4 ± 13.4
5 Weeks Posttreatment				
Total Protein (g/dL)	6.4 ± 0.1	6.7 ± 0.1	6.5 ± 0.1	6.6 ± 0.1
Albumin (g/dL)	3.7 ± 0.1	3.7 ± 0.1	3.7 ± 0.1	3.7 ± 0.1
AST (IU/L)	105.0 ± 2.0	90.0 ± 3.0	92.0 ± 5.0	90.0 ± 5.0
ALT (IU/L)	45.8 ± 1.3	44.0 ± 3.1	38.0 ± 1.9	37.4 ± 1.0
10 Weeks Posttreatment				
Total Protein (g/dL)	------[d]	------[d]	------[d]	------[d]
Albumin (g/dL)	3.9 ± <0.1	3.9 ± 0.1	3.8 ± 0.2	4.0 ± 0.1
AST (IU/L)	110.2 ± 10.8	82.6 ± 6.0	106.0 ± 29.1	76.6 ± 2.4
ALT (IU/L)	61.8 ± 6.5	41.2 ± 0.9	63.2 ± 22.5	42.2 ± 2.3

[a]Mean ± SEM, N=5.
[b]AST=aspartate aminotransferase, ALT=alanine aminotransferase
[c]Different from control at p<0.01.
[d]Parameter not evaluated at this posttreatment period.

TABLE 5.2-3. MEAN[a] PLASMA ACETYLCHOLINESTERASE VALUES IN RATS FOLLOWING DAILY GAVAGE TREATMENT WITH MIL-H-19457C

Treatment Time (Weeks)	Dose			
	Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
		Females		
3	1744.5 ± 162.6[b]	1179.6 ± 93.1	859.0 ± 90.6[c]	500.0 ± 38.5[c]
10	2202.4 ± 101.1	1365.4 ± 119.5	581.7 ± 38.1[c]	268.9 ± 30.7[c]
5 post	2638.4 ± 105.2	2567.2 ± 118.2	2198.4 ± 118.9	2268.4 ± 135.5
10 post	2657.1 ± 136.3	2273.8 ± 71.0	2272.4 ± 164.3	2569.3 ± 165.8
		Males		
3	490.7 ± 21.0	394.3 ± 29.6	344.4 ± 20.3[c]	337.7 ± 8.7[c]
10	452.3 ± 23.1	362.9 ± 15.0	322.9 ± 21.6[d]	243.4 ± 10.9[c]
9 post	433.6 ± 27.7	438.3 ± 27.4	445.8 ± 29.3	403.6 ± 18.3
18 post	439.0 ± 21.6	483.9 ± 28.3	451.6 ± 13.7	443.4 ± 21.5

[a]Mean ± SEM, N=5.
[b]N=4.
[c]Different from control at $p<0.01$.
[d]Different from control at $p<0.05$.

TABLE 5.2-4. ORGAN[a] WEIGHTS (g) AND ORGAN-TO-BODY-WEIGHT RATIOS (%) OF MALE FISCHER 344 RATS FOLLOWING REPEATED GAVAGE OF MIL-H-19457C HYDRAULIC FLUID

(3 Weeks Treatment)

	Dose			
Organ	Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Liver	7.35 ± 0.3	9.84 ± 0.3[b]	12.45 ± 0.6[b]	14.54 ± 0.6[b]
Ratio	3.09 ± 0.1	3.90 ±<0.1[b]	5.03 ± 0.1[b]	6.15 ±<0.1[b]
Kidney	1.88 ±<0.1	2.04 ± 0.1	2.12 ± 0.1	2.26 ± 0.1[c]
Ratio	0.79 ±<0.1	0.81 ±<0.1	0.86 ±<0.1[b]	0.96 ±<0.1[b]
Adrenal	0.04 ±<0.1	0.05 ±<0.1[b]	0.05 ±<0.1[b]	0.05 ±<0.1[b]
Ratio	0.02 ±<0.1	0.02 ±<0.1	0.02 ±<0.1	0.02 ±<0.1[b]
Left Testis	1.45 ±<0.1	1.43 ±<0.1	1.49 ±<0.1	1.44 ±<0.1
Ratio	0.61 ±<0.1	0.57 ±<0.1	0.61 ±<0.1	0.61 ±<0.1
Thymus	0.26 ±<0.1	0.31 ±<0.1	0.23 ±<0.1	0.29 ±<0.1
Ratio	0.11 ±<0.1	1.22 ±<0.1	0.09 ±<0.1	0.12 ±<0.1
Pituitary	0.01 ±<0.1	0.01 ±<0.1	0.01 ±<0.1	0.01 ±<0.1
Ratio	<0.01 ±<0.1	<0.01 ±<0.1	<0.01 ±<0.1	<0.01 ±<0.1
Left Epididymus	0.48 ±<0.1	0.46 ±<0.1	0.46 ±<0.1	0.42 ±<0.1
Ratio	0.20 ±<0.1	0.18 ±<0.1	0.18 ±<0.1	0.18 ±<0.1
Right Testis	1.15 ± 0.2	0.72 ± 0.3	1.41 ±<0.1	1.39 ±<0.1
Ratio	0.48 ± 0.1	0.28 ± 0.1	0.57 ±<0.1	0.59 ±<0.1
Right Cauda Epididymus	0.44 ± 0.2	0.91 ± 0.3	0.22 ±<0.1	0.20 ±<0.1
Ratio	0.19 ± 0.1	0.37 ± 0.1	0.09 ±<0.1	0.08 ±<0.1

[a]Mean ± SEM, N=5.
[b]Different from control at p<0.01.
[c]Different from control at p<0.05.

TABLE 5.2-5. ORGAN[a] WEIGHTS (g) AND ORGAN-TO-BODY-WEIGHT RATIOS (%) OF FEMALE FISCHER 344 RATS FOLLOWING REPEATED GAVAGE OF MIL-H-19457C HYDRAULIC FLUID
(3 Weeks Treatment)

	Dose			
Organ	Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Liver	4.52 ± 0.1	5.19 ± 0.2[c]	6.63 ± 0.1[b]	9.09 ± 0.4[b]
Ratio	2.83 ±<0.1	3.30 ± 0.1[b]	4.16 ± 0.1[b]	5.85 ± 0.3[b]
Kidney	1.24 ±<0.1	1.25 ± 0.1	1.34 ±<0.1	1.26 ±<0.1
Ratio	0.78 ±<0.1	0.79 ±<0.1	0.84 ±<0.1[c]	0.81 ±<0.1
Adrenal	0.05 ±<0.1	0.05 ±<0.1	0.06 ±<0.1[b]	0.06 ±<0.1[b]
Ratio	0.03 ±<0.1	0.03 ±<0.1	0.04 ±<0.1[b]	0.04 ±<0.1[b]
Ovaries	0.07 ±<0.1	0.09 ±<0.1	0.09 ±<0.1	0.08 ±<0.1
Ratio	0.04 ±<0.1	0.06 ±<0.1	0.06 ±<0.1	0.05 ±<0.1
Thymus	0.34 ±<0.1	0.30 ±<0.1	0.32 ±<0.1	0.30 ±<0.1
Ratio	0.21 ±<0.1	0.19 ±<0.1	0.20 ±<0.1	0.19 ±<0.1
Pituitary	0.01 ±<0.1	0.01 ±<0.1	0.01 ±<0.1	0.01 ±<0.1
Ratio	0.01 ±<0.1	0.01 ±<0.1	0.01 ±<0.1	0.01 ±<0.1

[a]Mean ± SEM, N=5.
[b]Different from control at p<0.01.
[c]Different from control at p<0.05.

TABLE 5.2-6. ORGAN[a] WEIGHTS (g) AND ORGAN-TO-BODY-WEIGHT RATIOS (%) OF MALE FISCHER 344 RATS FOLLOWING REPEATED GAVAGE OF MIL-H-19457C HYDRAULIC FLUID

(10 Weeks Treatment)

	Dose			
Organ	Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Liver	9.82 ± 0.5	12.81 ± 1.0	13.80 ± 0.6[b]	17.39 ± 0.6[b]
Ratio	2.93 ± 0.1	3.80 ± 0.1[b]	4.53 ± 0.1[b]	6.20 ± 0.1[b]
Kidney	2.39 ± 0.1	2.38 ± 0.1	2.50 ± 0.1	2.72 ± 0.1
Ratio	0.71 ±<0.1	0.75 ±<0.1	0.82 ±<0.1[b]	0.97 ±<0.1[b]
Adrenal	0.06 ±<0.1	0.06 ±<0.1	0.05 ±<0.1	0.07 ±<0.1
Ratio	0.02 ±<0.1	0.02 ±<0.1	0.02 ±<0.1	0.02 ±<0.1[b]
Left Testis	1.53 ±<0.1	1.46 ±<0.1	1.52 ±<0.1	1.55 ±<0.1
Ratio	0.46 ±<0.1	0.46 ±<0.1	0.50 ±<0.1	0.55 ±<0.1[b]
Thymus	0.35 ±<0.1	0.31 ±<0.1	0.27 ±<0.1[c]	0.23 ±<0.1[b]
Ratio	0.11 ±<0.1	0.10 ±<0.1	0.09 ±<0.1	0.08 ±<0.1
Pituitary	0.01 ±<0.1	0.01 ±<0.1	0.01 ±<0.1	0.01 ±<0.1
Ratio	<0.01 ±<0.1	<0.01 ±<0.1	<0.01 ±<0.1	<0.01 ±<0.1
Left Epididymus	0.51 ±<0.1	0.49 ±<0.1	0.50 ±<0.1	0.47 ±<0.1
Ratio	0.15 ±<0.1	0.16 ±<0.1	0.16 ±<0.1	0.17 ±<0.1
Right Testis	1.49 ± 0.1	1.44 ±<0.1	1.58 ±<0.1	1.51 ±<0.1
Ratio	0.45 ± 0.1	0.46 ±<0.1	0.52 ±<0.1[c]	0.54 ±<0.1[c]
Right Cauda Epididymus	0.25 ±<0.1	0.22 ±<0.1	0.25 ±<0.1	0.24 ±<0.1
Ratio	0.07 ±<0.1	0.07 ±<0.1	0.08 ±<0.1	0.09 ±<0.1

[a]Mean ± SEM, N=5.
[b]Different from control at p<0.01.
[c]Different from control at p<0.05.

TABLE 5.2-7. ORGAN[a] WEIGHTS (g) AND ORGAN-TO-BODY-WEIGHT RATIOS (%) OF FEMALE FISCHER 344 RATS FOLLOWING REPEATED GAVAGE OF MIL-H-19457C HYDRAULIC FLUID
(10 Weeks Treatment)

	Dose			
Organ	Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Liver	4.88 ± 0.1	5.43 ± 0.1[b]	7.49 ± 0.1[b]	10.90 ± 0.4[b]
Ratio	2.64 ± 0.1	3.09 ±<0.1[b]	4.31 ±<0.1[b]	6.10 ± 0.2[b]
Kidney	1.35 ±<0.1	1.33 ±<0.1	1.39 ±<0.1	1.53 ±<0.1[b]
Ratio	0.73 ±<0.1	0.76 ±<0.1	0.80 ±<0.1[b]	0.85 ±<0.1[b]
Adrenal	0.06 ±<0.1	0.06 ±<0.1	0.06 ±<0.1	0.07 ±<0.1[b]
Ratio	0.03 ±<0.1	0.03 ±<0.1	0.04 ±<0.1	0.04 ±<0.1[b]
Ovaries	0.09 ±<0.1	0.08 ±<0.1	0.08 ±<0.1	0.09 ±<0.1
Ratio	0.05 ±<0.1	0.05 ±<0.1	0.05 ±<0.1	0.05 ±<0.1
Thymus	0.28 ±<0.1	0.26 ±<0.1	0.22 ±<0.1[c]	0.27 ±<0.1
Ratio	0.15 ±<0.1	0.15 ±<0.1	0.13 ±<0.1	0.15 ±<0.1
Pituitary	0.02 ±<0.1	0.01 ±<0.1	0.01 ±<0.1	0.01 ±<0.1
Ratio	0.01 ±<0.1	0.01 ±<0.1	0.01 ±<0.1	0.01 ±<0.1

[a]Mean ± SEM, N=5.
[b]Different from control at p<0.01.
[c]Different from control at p<0.05.

TABLE 5.2-8. ORGAN[a] WEIGHTS (g) AND ORGAN-TO-BODY-WEIGHT RATIOS (%) OF FEMALE FISCHER 344 RATS FOLLOWING REPEATED GAVAGE OF MIL-H-19457C HYDRAULIC FLUID
(5 Weeks Posttreatment)

	Dose			
Organ	Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Liver	5.24 ± 0.2	5.35 ± 0.3	5.47 ± 0.2	6.05 ± 0.2
Ratio	2.62 ± 0.1	2.77 ± 0.1	2.81 ± 0.1[b]	3.05 ± 0.1[b]
Kidney	1.42 ±<0.1	1.41 ±<0.1	1.40 ±<0.1	1.47 ±<0.1
Ratio	0.71 ±<0.1	0.73 ±<0.1	0.72 ±<0.1	0.74 ±<0.1
Adrenal	0.05 ±<0.1	0.06 ±<0.1	0.05 ±<0.1[c]	0.06 ±<0.1[b]
Ratio	0.03 ±<0.1	0.03 ±<0.1	0.03 ±<0.1[b]	0.03 ±<0.1[b]
Ovaries	0.07 ±<0.1	0.09 ±<0.1	0.09 ±<0.1	0.09 ±<0.1
Ratio	0.04 ±<0.1	0.05 ±<0.1	0.04 ±<0.1	0.04 ±<0.1
Thymus	0.19 ±<0.1	0.22 ±<0.1	0.24 ±<0.1	0.22 ±<0.1
Ratio	0.10 ±<0.1	0.11 ±<0.1	0.13 ±<0.1[c]	0.11 ±<0.1
Pituitary	0.02 ±<0.1	0.02 ±<0.1	0.02 ±<0.1	0.02 ±<0.1
Ratio	0.01 ±<0.1	0.01 ±<0.1	0.01 ±<0.1	0.01 ±<0.1

[a]Mean ± SEM, N=5.
[b]Different from control at p<0.01.
[c]Different from control at p<0.05.

TABLE 5.2-9. ORGAN[a] WEIGHTS (g) AND ORGAN-TO-BODY-WEIGHT RATIOS (%) OF FEMALE FISCHER 344 RATS FOLLOWING REPEATED GAVAGE OF MIL-H-19457C HYDRAULIC FLUID
(10 Weeks Posttreatment)

	Dose			
Organ	Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Liver	5.33 ± 0.2	5.27 ± 0.1	5.54 ± 0.1	5.58 ±<0.1
Ratio	2.64 ±<0.1	2.72 ±<0.1	2.79 ±<0.1	2.81 ±<0.1
Kidney	1.43 ±<0.1	1.42 ±<0.1	1.46 ±<0.1	1.50 ±<0.1
Ratio	0.02 ±<0.1	0.03 ±<0.1	0.02 ±<0.1	0.02 ±<0.1
Adrenal	0.06 ±<0.1	0.06 ±<0.1	0.06 ±<0.1[b]	0.07 ±<0.1[c]
Ratio	0.03 ±<0.1	0.03 ±<0.1	0.03 ±<0.1[b]	0.03 ±<0.1[b]
Ovaries	0.08 ±<0.1	0.09 ±<0.1	0.10 ±<0.1	0.09 ±<0.1
Ratio	0.04 ±<0.1	0.05 ±<0.1	0.05 ±<0.1[c]	0.05 ±<0.1
Thymus	0.20 ±<0.1	0.21 ±<0.1	0.20 ±<0.1	0.17 ±<0.1
Ratio	0.10 ±<0.1	0.10 ±<0.1	0.10 ±<0.1[c]	0.10 ±<0.1
Pituitary	0.01 ±<0.1	0.01 ±<0.1	0.01 ±<0.1	0.01 ±<0.1
Ratio	0.01 ±<0.1	0.01 ±<0.1	0.01 ±<0.1	0.01 ±<0.1

[a]Mean ± SEM, N=5.
[b]Different from control at p<0.01.
[c]Different from control at p<0.05.

TABLE 5.2-10. ORGAN[a] WEIGHTS (g) AND ORGAN-TO-BODY-WEIGHT RATIOS (%) OF MALE FISCHER 344 RATS FOLLOWING REPEATED GAVAGE OF MIL-H-19457C HYDRAULIC FLUID

(9 Weeks Posttreatment)

	Dose			
Organ	Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Liver	11.36 ± 0.4	11.73 ± 0.5	10.88 ± 0.2	12.34 ± 0.3
Ratio	2.95 ± <0.1	3.03 ± <0.1	3.10 ± 0.1	3.17 ± <0.1
Kidneys	2.79 ± 0.1	2.70 ± 0.1	2.46 ± 0.1	2.86 ± 0.1
Ratio	0.72 ± <0.1	0.70 ± <0.1	0.70 ± <0.1	0.74 ± <0.1
Adrenal	0.05 ± <0.1	0.06 ± <0.1	0.06 ± <0.1	0.05 ± <0.1
Ratio	0.01 ± <0.1	0.02 ± <0.1	0.02 ± <0.1	0.01 ± <0.1
Left Testis	1.52 ± <0.1	1.55 ± 0.1	1.49 ± <0.1	1.61 ± 0.1
Ratio	0.40 ± <0.1	0.40 ± <0.1	0.42 ± <0.1	0.41 ± <0.1
Thymus	0.31 ± <0.1	0.29 ± <0.1	0.29 ± <0.1	0.29 ± <0.1
Ratio	0.08 ± <0.1	0.08 ± <0.1	0.08 ± <0.1	0.08 ± <0.1
Pituitary	0.01 ± <0.1	0.01 ± <0.1	0.01 ± <0.1	0.01 ± <0.1
Ratio	<0.01 ± <0.1	<0.01 ± <0.1	<0.01 ± <0.1	<0.01 ± <0.1
Left Epididymus	0.50 ± <0.1	0.54 ± <0.1	0.48 ± <0.1	0.55 ± <0.1
Ratio	0.13 ± <0.1	0.14 ± <0.1	0.14 ± <0.1	0.14 ± <0.1
Right Testis	1.49 ± <0.1	1.53 ± <0.1	1.49 ± <0.1	1.55 ± <0.1
Ratio	0.39 ± <0.1	0.40 ± <0.1	0.43 ± <0.1	0.40 ± <0.1
Right Cauda Epididymus	0.26 ± <0.1	0.26 ± <0.1	0.24 ± <0.1	0.27 ± <0.1
Ratio	0.07 ± <0.1	0.07 ± <0.1	0.07 ± <0.1	0.07 ± <0.1

[a]Mean ± SEM, N=5.

TABLE 5.2-11. ORGAN[a] WEIGHTS (g) AND ORGAN-TO-BODY-WEIGHT RATIOS (%) OF MALE FISCHER 344 RATS FOLLOWING REPEATED GAVAGE OF MIL-H-19457C HYDRAULIC FLUID
(18 Weeks Posttreatment)

	Dose			
Organ	Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Liver	12.47 ± 0.8	12.36 ± 0.4	12.04 ± 0.3	11.76 ± 0.4
Ratio	2.85 ± 0.1	2.79 ± 0.1	2.80 ± 0.1	2.81 ± 0.1
Kidneys	3.08 ± 0.2	2.99 ± 0.1	3.01 ± <0.1	3.01 ± 0.1
Ratio	0.71 ± <0.1	0.68 ± <0.1	0.70 ± <0.1	0.72 ± <0.1
Adrenal	0.06 ± <0.1	0.06 ± <0.1	0.05 ± <0.1	0.06 ± <0.1
Ratio	0.01 ± <0.1	0.01 ± <0.1	0.01 ± <0.1	0.01 ± <0.1
Left Testis	1.56 ± 0.1	1.66 ± <0.1	1.58 ± 0.1	1.66 ± <0.1
Ratio	0.36 ± <0.1	0.38 ± <0.1	0.37 ± <0.1	0.40 ± <0.1
Thymus	0.25 ± <0.1	0.25 ± <0.1	0.21 ± <0.1	0.22 ± <0.1
Ratio	0.06 ± <0.1	0.06 ± <0.1	0.05 ± <0.1	0.05 ± <0.1
Pituitary	0.01 ± <0.1	0.01 ± <0.1	0.01 ± <0.1	0.01 ± <0.1
Ratio	<0.01 ± <0.1	<0.01 ± <0.1	<0.01 ± <0.1	<0.01 ± <0.1
Left Epididymus	0.50 ± <0.1	0.52 ± <0.1	0.49 ± <0.1	0.53 ± <0.1
Ratio	0.12 ± <0.1	0.12 ± <0.1	0.12 ± <0.1	0.13 ± <0.1
Right Testis	1.43 ± 0.2	1.58 ± 0.1	1.54 ± 0.1	1.58 ± 0.1
Ratio	0.33 ± <0.1	0.35 ± <0.1	0.36 ± <0.1	0.38 ± <0.1
Right Cauda Epididymus	0.25 ± <0.1	0.30 ± <0.1	0.27 ± <0.1	0.26 ± <0.1
Ratio	0.06 ± <0.1	0.07 ± <0.1	0.06 ± <0.1	0.06 ± <0.1

[a]Mean ± SEM, N=5.

Sperm was analyzed at each male sacrifice using a videomicrography system (CellSoft Automated Semen Analyzer, Cryo Resources, Ltd.) However, after the study was completed and the results had been recorded on videotape, it was determined that the system was malfunctioning. A CellSoft representative determined that the parameters preset in the system were incorrect and were not those needed to analyze rat semen. In addition, improper equipment had been substituted for the original equipment on the microscope set-up. All of these problems have resulted in the loss of these data.

There are a number of parameters yet to be analyzed, including estradiol levels in blood and histopathology.

REFERENCES

Carlton, B.D., A.H. Basaran, L.E. Mezza, and M.K. Smith. 1987. Examination of the reproductive effects of tricresyl phosphate administered to Long-Evans rats. *Toxicology* 46:321-328.

Chapin, R.E., J.D. George, and J.C. Lamb. 1988. Reproductive toxicity of tricresylphosphate in a continuous breeding protocol in Swiss (CD-1) mice. *Fundam. Appl. Toxicol.* 10:344-354.

Doull, J.D., C.D. Klaassen, and M.D. Amdur, eds. 1979. *Casarett and Doull's Toxicology: The Basic Science of Poisons*. New York, NY: MacMillan.

Dunnick, J.K., B.N. Gupta, M.W. Harris, and J.C. Lamb. 1984. Reproductive toxicity of dimethyl methyl phosphonate in the male Fischer 344 rat. *Toxicol. Appl. Pharmacol.* 72:379-387.

Gaworski, C.L., E.R. Kinkead, J.R. Horton, W.J. Bashe, R.L. Einhaus, D.L. Pollard, J.D. Diaz, R.A. Salomon, T.R. Boosinger, R.H. Bruner, and A.P. D'Addario. 1986. Comparative studies of the short-term toxicity of the hydraulic fluids MIL-H-19457C, MIL-H-19457B, and MIL-H-22072B. AAMRL-TR-86-030, Wright-Patterson Air Force Base, OH: Armstrong Aerospace Medical Research Laboratory.

Hanna, P.J. and J.B. Kerr. 1981. Antifertility activity of trimethylphosphate in male rats. *Experientia* 37:999-1001.

Harbison, R.D., C. Dwivedi, and M.A. Evans. 1976. A proposed mechanism for trimethylphosphate-induced sterility. *Toxicol. Appl. Pharmacol.* 35:481-490.

Hejtmancik, M., R. Derkin, M. Ryan, B. Carlton, G. Haggerty, A. Peters, and R. Irwin. 1986. Comparative dosed feed and gavage subchronic studies of tricresyl phosphate (TCP) in F344 rats and B6C3F1 mice. *The Toxicologist*, Abstracts of the 25th Anniversary Meeting (#832) 6:207.

Kinkead, E.R., S.K. Bunger, R.E. Wolfe, W.T. Brashear, and J.R. Latendresse. 1991. Dermal absorption and target organ toxicity of H-19457C hydraulic fluid. AL-TR-1991-0108, NMRI-91-61. Wright-Patterson Air Force Base, OH: Armstrong Laboratory and Navy Medical Research Institute.

Kinkead, E.R., R.E. Wolfe, and S.A. Salins. 1992. Determination of dose-response effect and no-effect levels following gavage of tricresylphosphate and MIL-H-19457C hydraulic fluid. In: H.G. Wall, D. Dodd, A. Vinegar, H. Clewell, eds. *1991 Toxic Hazards Research Unit Annual Report*, pp. 179-185. AL-TR-1992-0142/NMRI-92-97. Wright-Patterson Air Force Base, OH: Armstrong Laboratory and Naval Medical Research Institute.

Kinkead, E.R., R.E. Wolfe, and S.A. Salins. 1993. Determination of dose-response effect and no observable effect levels in Fischer 344 rats following repeated gavage of tricresylphosphate and MIL-H-19457C hydraulic fluid. In: H.G. Wall, D.E. Dodd, A. Vinegar, and M.G. Schneider, eds. *1992 Toxic Hazards Research Unit Annual Report*, pp. 238-250. AL/OE-TR-1993-0105, NMRI-93-33. Wright-Patterson Air Force Base, OH: Armstrong Laboratory and Naval Medical Research Institute.

Krause, W. 1977. Influence of DDT, DDVP, and malathion on FSH, LH, and testosterone serum levels and testosterone concentration in testis. *Bull. Environ. Contam. Toxicol.* 18:231-242.

Latendresse, J.R., S. Azhar, C.L. Brooks, and C.C. Capen. 1993. Pathogenesis of cholesteryl lipidosis in adrenal cortocal and ovarian interstitial cells in F344 rats caused by tricresyl phosphate (TCP) and butylated triphenyl phosphate (BTP). *Toxicol. Appl. Pharmacol.* 122:281-289.

Latendresse, J.R., C.C. Capen, and C.L. Brooks. 1992a. Pathologic effects of butylated triphenyl phosphate-based hydraulic fluid (BTP) on the adrenal gland, ovary, and testis in the Fischer 344 rat. In: *Endocrine and Reproductive Toxicity of Butylated Triphenyl Phosphate and Tricresyl Phosphate in the F344 Rat,* pp. 1-64. University Microfilms International.

Latendresse, J.R., C.C. Capen, and C.L. Brooks. 1992b. The toxic effects of butylated triphenyl phosphate-based hydraulic fluid and tricresyl phosphate on ovarian, adrenocortical, and liver function in the female F344 rat. In: *Endocrine and Reproductive Toxicity of Butylated Triphenyl Phosphate and Tricresyl Phosphate in the F344 Rat,* pp. 65-107. University Microfilms International.

Latendresse, J.R., C.C. Capen, and C.L. Brooks. 1992c. Comparison of reproductive toxicity of butylated triphenyl phosphate (BTP) and tricresyl phosphate (TCP) fluids in F344 rats. In: *Endocrine and Reproductive Toxicity of Butylated Triphenyl Phosphate and Tricresyl Phosphate in the F344 Rat,* pp. 108-140. University Microfilms International.

Oishi, H., S. Oishi, and K. Hiraga. 1982. Toxicity of several phosphoric acid esters in rats. *Toxicol. Lett.* 13:29-34.

Toy, A.D.F. 1977. Miscellaneous industrial use for inorganic phosphorus compounds. In: *Phosphorus Chemistry in Everyday Living*, pp 152-157. American Chemical Society.

Wall, H.G., C.L. Gaworski, E.R. Kinkead, A. Vinegar, R.H. Bruner, and C.D. Flemming. 1990. Evaluation of the 90-day continuous inhalation toxicity of Fyrquel 220, Durad MP280, and Houghto-Safe 273. Letter report, 26 January 1990. Wright-Patterson Air Force Base, OH: Armstrong Aerospace Medical Research Laboratory.

5.3 RESULTS OF LUNG ORGAN WEIGHTS IN SUPPORT OF THE RESEARCH ON THE PULMONARY EFFECTS OF TOXIC DUST AND SMOKE INHALATION: MUSTARD GAS AND DUSTY MUSTARD SURROGATES

E.R. Kinkead, E.C. Kimmel[1], J.E. Reboulet[1], and D.L. Pollard

ABSTRACT

This study evaluated the inhalation toxicity of a complex mixture of gases in a dusty high-particulate atmosphere. These exposures were conducted to evaluate the inhalation toxicity of acrolein and acrolein/particulate to serve as a surrogate of similar exposures to mustard gas and its particulate counterpart "dusty mustard." The results reported herein describe the activities tasked to the Toxic Hazards Research Unit, which were the wet/dry lung evaluations performed on exposed animals at various time points following exposure. Lung water expressed as a ratio to body weight was significantly increased in rats exposed to 10 ppm acrolein when compared to control animals. Lung water was not increased in animals inhaling the acrolein/particulate atmospheres.

INTRODUCTION

There is an interest in the comprehensive study of the pulmonary effects of vesicant chemical warfare (CW) agents such as mustard gas (HD). Mortality produced by HD is low with virtually all fatalities attributed to pulmonary effects. The most common effects of HD exposure are debilitating acute respiratory complications such as acute tracheobronchitis pneumonia and adult respiratory distress syndrome.

The literature contains little information on the mechanism or pathogenesis of HD-induced lung damage. However, evidence suggests that inhalation of HD initially results in acute respiratory irritation followed by pulmonary edema accompanied by dyspnea, leading to obstructive pulmonary disease. This pattern is similar to that which occurs following exposure to other known chemical toxins, such as acrolein (Costa et al., 1986), cigarette smoke (Kimmel et al., 1985), and smoke from combustion atmospheres (Hales et al., 1988). Acrolein is a major component in many toxic combustion smokes (Beauchamp et al., 1985).

Complicating the characterization of the mechanisms and pathogenesis of HD-induced injury is the fact that in aerosol form or other mixture forms such as HD absorbed on silica gel powder (dusty mustard), pulmonary deposition will be different with more severe lung lesions and probably shortened pathogenesis. Mustard gas is absorbed primarily in the upper respiratory tract with little HD penetrating to the pulmonary

---

[1]Current address: Geo-Centers, Inc., NMRI/TD, Wright-Patteson AFB, OH.

parenchyma of the deep lung. Studies with other lung irritants, including acrolein, have shown that toxic gases or vapors that do not normally reach the lower lung may do so if absorbed into aerosol particles. These complex atmospheres may produce physiological effects not induced by the vapor or aerosol particle alone (Boren, 1964; Kilburn and McKenzie, 1978).

MATERIALS AND METHODS

The materials and methods of this study were reported by Kimmel (1992). The particle morphology, particle concentration and distribution, as well as aerosol ammonium dinitramide particle generation characteristics were reported by Reboulet et al. (1993). The Toxic Hazards Research Unit's final involvement in this project, upon completion of the exposure system, was to coordinate animal logistics and to measure the wet and dry lung weights of representative animals from interim sacrifices following exposure.

RESULTS

The rats exposed to 10 ppm acrolein showed severe respiratory distress following exposure, and none survived beyond 24 h. Rats necropsied at 1, 4, and 24 h postexposure demonstrated severe pulmonary edema as evidenced by increased lung water, grams water per kilogram body weight, and grams solids per kilogram body weight (Table 5.3-1). All of these parameters were significantly ($p < 0.01$) increased when compared to the control or other exposed groups. The lung water expressed as a ratio to body weight was increased approximately 38% when compared to control values. None of the other exposures showed an increase in lung water weight at any of the time points examined.

Other parameters examined following necropsy included tissues for light and electron microscopy evaluations. The Triservices pathology section is preparing a final report, which will be provided to the Naval Medical Research Institute's (NMRI's) Principle Investigator. A final report detailing all events from this research project will be prepared by the NMRI Principle Investigator.

TABLE 5.3-1. RESULTS OF WET/DRY LUNG WEIGHTS FOLLOWING INHALATION FOR FOUR HOURS[a].

Parameter[b]	Time Posttreatment 1 h	4 h	24 h	3 days	7 days	14 days
750 mg/m³ Aerosol						
Lung H_2O (%)	75.1 ± 0.9	75.4 ± 1.0	76.4 ± 0.5	75.7 ± 0.7	76.2 ± 0.6	75.9 ± 0.6
Lung H_2O/kg	3.1 ± 0.2	3.1 ± 0.2	3.3 ± 0.1	2.9 ± 0.1	2.9 ± 0.1	2.7 ± <0.1
Lung solid/kg	1.0 ± <0.1	1.0 ± <0.1	1.0 ± <0.1	0.9 ± <0.1	0.9 ± <0.1	0.9 ± <0.1
2.5 ppm						
Lung H_2O (%)	73.2 ± 1.2	75.6 ± 1.1	75.0 ± 0.9	76.6 ± 0.5	76.9 ± 0.4	75.9 ± 0.4
Lung H_2O/kg	2.8 ± 0.1	3.1 ± 0.2	2.8 ± 0.1	3.0 ± <0.1	3.0 ± <0.1	2.8 ± <0.1
Lung solid/kg	1.0 ± <0.1	1.0 ± <0.1	0.9 ± <0.1	0.9 ± <0.1	0.9 ± <0.1	0.9 ± <0.1
10 ppm						
Lung H_2O (%)	79.7 ± 0.2[c]	79.0 ± 0.6[c]	76.6 ± 0.3	----	----	----
Lung H_2O/kg	4.0 ± 0.1[c]	4.1 ± 0.2[c]	4.1 ± 0.2[c]	----	----	----
Lung solid/kg	1.0 ± <0.1[c]	1.1 ± <0.1[c]	1.3 ± <0.1[c]	----	----	----
Control						
Lung H_2O (%)	74.9 ± 0.7	76.7 ± 0.8	74.3 ± 0.5	76.0 ± 0.3	75.1 ± 0.5	76.2 ± 0.3
Lung H_2O/kg	2.9 ± 0.1	3.1 ± 0.1	2.7 ± 0.1	2.8 ± 0.1	2.8 ± 0.1	2.9 ± <0.1
Lung solid/kg	1.0 ± <0.1	0.9 ± <0.1	0.9 ± <0.1	0.9 ± <0.1	0.9 ± <0.1	
2.5 ppm + Aerosol						
Lung H_2O (%)	75.4 ± 0.5	76.0 ± 0.5	76.6 ± 0.2	75.3 ± 0.6	75.4 ± 0.7	75.9 ± 0.7
Lung H_2O/kg	2.9 ± 0.1	3.0 ± 0.1	3.0 ± 0.1	2.8 ± 0.1	2.8 ± 0.1	2.7 ± <0.1
Lung solid/kg	0.9 ± <0.1	1.0 ± <0.1	0.9 ± <0.1	0.9 ± <0.1	0.9 ± <0.1	0.9 ± <0.1
5 ppm						
Lung H_2O (%)	75.5 ± 0.7	75.8 ± 0.7	75.0 ± 0.9	77.6 ± 0.3	76.9 ± 0.2	75.1 ± 0.7
Lung H_2O/kg	2.9 ± 0.1	2.9 ± 0.1	2.9 ± 0.8	3.2 ± 0.1	3.0 ± 0.1	2.6 ± 0.2
Lung solid/kg	0.9 ± <0.1	0.9 ± <0.1	1.0 ± <0.1	0.9 ± <0.1	0.9 ± <0.1	0.9 ± <0.1
5 ppm + Aerosol						
Lung H_2O (%)	76.0 ± 0.4	76.2 ± 0.3	74.9 ± 0.3	75.9 ± 0.6	77.0 ± 0.3	76.6 ± 1.1
Lung H_2O/kg	2.9 ± 0.1	3.0 ± 0.1	2.8 ± 0.1	2.9 ± 0.1	3.1 ± 0.2	3.0 ± 0.2
Lung solid/kg	0.9 ± <0.1	0.9 ± <0.1	0.9 ± <0.1	0.9 ± <0.1	0.9 ± <0.1	0.9 ± <0.1

[a]Mean ± SEM, N=3.
[b]Lung H_2O (%) = (wet weight - dry weight)/wet weight x 10^3.
Lung H_2O/kg = (wet weight - dry weight)/body weight (g) x 10^3.
Lung solid/kg body weight = dry weight/body weight (g) x 10^3.
[c]Significantly different than control and other groups at $p<0.01$.

REFERENCES

Beauchamp, R.O., Jr., D.A. Andjelkovich, A.D. Kligerman, K.T. Morgan, and H. d'A Heck. 1985. A critical review of the literature on acrolein toxicity. *CRC Crit Rev. Toxicol.* 14:309-380.

Boren, H.G. 1964. Carbon as a carrier mechanism for irritant gases. *Am. Ind. Hyg. Assoc. J.* 41:888-894.

Costa, D.L., R.S. Kutzman, J.R. Lehman, and R.T. Drew. 1986. Altered lung function and structure in the rat after subchronic exposure to acrolein. *Am. Rev. Respir. Dis.* 133:286-291.

Hales, C.A., P.W. Barkin, W. Jung, E. Trautman, D. Lamborghini, N. Herrig, and J. Burke. 1988. Synthetic smoke with acrolein but not HCl produces pulmonary edema. *J. Appl. Physiol.* 64:1121-1133.

Kilburn, R.N. and W.N. McKenzie. 1978. Leucocyte recruitment to airways by aldehyde-carbon combinations that mimic cigarette smoke. *Lab. Invest.* 38:134-143.

Kimmel, E.C., D.W. Winsett, and L. Diamond. 1985. The augmentation of elastase-induced emphysema by cigarette smoke: Description of a model and review of possible mechanisms. *Am. Rev. Respir. Dis.* 132:885-894.

Kimmel, E.C. 1992. Lung response to toxic dust and smoke inhalation. In: H.G. Wall, D.E. Dodd, A. Vinegar, and H.J. Clewell III, eds. *1991 Toxic Hazards Research Unit Annual Report*, pp. 225-230. AL-TR-1992-0142, NMRI-92-97. Wright-Patterson Air Force Base, OH: Armstrong Laboratory and Naval Medical Research Institute.

5.4 ANALYSIS OF THE METABOLISM OF METHYLENE CHLORIDE IN THE B6C3F$_1$ MOUSE AND ITS IMPLICATIONS FOR HUMAN CARCINOGENIC RISK

H.J. Clewell III[1], J.M. Gearhart[1], and M.E. Andersen[2]

INTRODUCTION

The physiologically based pharmacokinetic (PBPK) model of Andersen et al. (1987), which was used by the U.S. Environmental Protection Agency (EPA) as the basis for their methylene chloride (MC) risk assessment, was actually a minor modification of a more extensive model that had previously been developed (Gargas et al., 1986b) to study the kinetics of dihalomethanes. The dihalomethane model is capable of predicting the time-course of the parent chemical, as well as the production of metabolites by both a glutathione transferase (GST) pathway, which is linear in the physiologically relevant range, and a saturable, mixed function oxidase (MFO) pathway. The model also is capable of describing the production of carbon monoxide (CO) during the oxidative metabolism and its subsequent binding to hemoglobin in the blood to produce carboxyhemoglobin (HbCO), which can be measured in experiments to test the model's predictions for saturable metabolism. The model provides a coherent description of data from both rodents and humans for several routes of exposure and a number of different chemicals.

In our previous submission to the Occupational Safety and Health Administration (OSHA), a sensitivity analysis and a Monte Carlo uncertainty analysis were performed on two alternative formulations of the MC risk assessment model, to estimate the impact of uncertainty regarding the model parameters on the calculation of human risk. Figure 5.4-1 displays the results of the analysis of the sensitivity of the risks calculated by the MC model to the parameters used in the model. Note that although the model contains more than two dozen parameters (see Appendix A for definitions), only nine of these have sufficient impact on the predicted risks to merit being displayed in the figure. Of those displayed, none have normalized sensitivities significantly greater than 1 (which would indicate amplification of error). Of the many parameters in the model, only the blood/air partition coefficients, the alveolar ventilation rates, and the metabolic parameters play a significant role in the determination of risk. The Monte Carlo analysis demonstrated that the uncertainty in the model parameters did produce a corresponding uncertainty in the PBPK-derived risk estimates. A methodology was proposed for incorporating an estimate of parameter uncertainty into the PBPK risk assessment by using the mean and upper 95th percentile of the distribution of risk estimates from the Monte Carlo simulations. The purpose of the current study was to refine the PBPK-derived risk assessment for MC by conducting laboratory experiments to characterize the key parameters in the model: the partition coefficients and the metabolic parameters.

---

[1]Current address: K.S. Crump Group, ICF Kaiser International, Ruston, LA.

[2]HERL, U.S. Environmental Protection Agency, Research Triangle Park, NC.

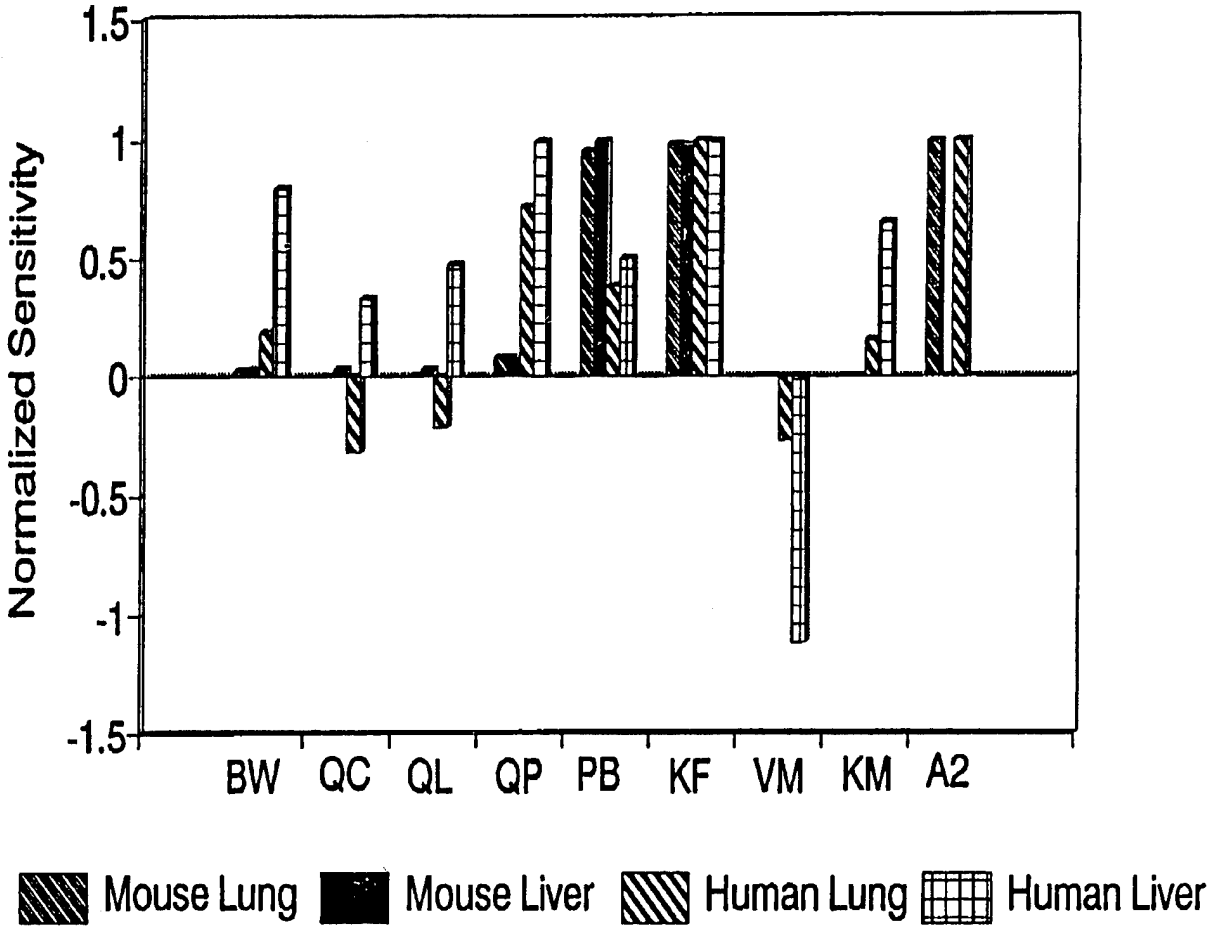


Figure 5.4-1. Analytical Sensitivities of Methylene Chloride Model Parameters for the Glutathione-*S*-Transferase Pathway Dose Surrogate. Parameter abbreviations are explained in Appendix A.

The initial Monte Carlo analysis used coefficients of variation for the partition coefficients based on results obtained with perchloroethylene. In the current study, repeated measurements of tissue/air and blood/air partition coefficients for mice, along with blood/air partition coefficients for humans, were performed using MC. These new measurements provided the basis for the partition coefficient estimates and coefficients of variation used in a new Monte Carlo analysis.

The initial analysis also noted that there was significant disagreement between different laboratories as to the appropriate parameters to characterize MC metabolism in the mouse. In order to shed light on this question, a series of closed-chamber experiments were performed using both MC and its deuterated analogue (DMC). Closed-chamber experiments also were performed in which oxidative metabolism was inhibited using *trans*-1,2-dichloroethylene (TRANS) prior to exposing mice to MC or DMC. In all cases, studies were performed on both male and female B6C3F$_1$ mice; however, only female results will be reported here because

the female B6C3F$_1$ mouse was the sex/strain yielding the highest carcinogenic potency in the MC bioassay. Studies were conducted on the male mouse primarily to determine whether sex differences in pharmacokinetics should be considered in the interpretation of the bioassay. The refined estimates for the metabolic parameters in the female mouse were then used in the new Monte Carlo analysis.

MATERIALS AND METHODS

Animals

Male B6C3F$_1$ mice (Charles Rivers, Wilmington, MA) weighing between 26 and 35 g and under 6 months of age were used in this study. All animals were maintained under standard conditions of light (6:00 a.m. to 6:00 p.m.) and temperature (20 to 23°C). Purina Formulab #5008 (Ralston Purina, St. Louis, MO) and water were available ad libitum until the time of exposure.

Determination of Tissue and Blood Partition Coefficients

A variation of the vial equilibrium method of Gargas et al. (1989) was used to determine MC blood/air or tissue/air partition coefficients in individual animals. Fresh tissues were homogenized and then ≈ 200 mg of blood, muscle, liver, lung, brain, kidney, or fat was smeared on the wall of a 9-mL liquid scintillation vial. Each vial was then injected with MC from a standard bag and incubated for 3.5 hours at 37 °C. A headspace sample (1 mL) was injected onto a gas chromatograph (GC) equipped with a flame ionization detector.

Closed-Chamber Analysis

Groups of five mice were exposed to MC using a closed recirculating system similar to that described by Gargas et al. (1986a), but with a chamber volume of 2.5 L. Initial MC concentrations were 200, 500, 1000, 1500, 2000, 3500, or 5000 ppm. Chamber MC concentration was monitored every 5 min for the first 30 min and every 15 min thereafter, using a gas sampling valve connected to a GC (Hewlett-Packard, Palo Alto, CA). Chromatography was performed on a 12 ft, 1/8 in. stainless steel column packed with 10% SE30 on 80/100 mesh Chromosorb W-HP (Supelco, Bellfonte, PA). This same procedure and concentrations were repeated with the same number of mice, except using DMC instead of the protonated material. Real-time CO chamber concentrations were determined for select MC concentrations with an electrochemical sensor specific for CO (Sieger Limited, Lincolnshire, IL). In the studies involving TRANS pretreatment, groups of five mice were initially exposed for 1.5 h to TRANS at an initial concentration of 100 ppm, using the closed recirculating system. The TRANS-pretreated animals were then exposed to initial MC concentrations of 500, 1000, or 2000 ppm and the chemical uptake rates were determined.

Determination of Carboxyhemoglobin

At the end of gas uptake exposures, mice were removed from the chamber and a 3-μL blood sample was taken from the lateral tail vein. The sample was pipetted into a quartz cuvette containing sodium hydrosulfite and the HbCO was determined by the method of Rodkey et al. (1979).

RESULTS

Partition Coefficients

The results of the partition coefficient determinations for female mice are shown in Table 5.4-1. Similar results were obtained for male mice (not shown). In general, tissue/air partition coefficients were reproducible within 10 to 30% and blood/air partition coefficients were reproducible within 10%. Additional uncertainty was assumed for partition coefficients of tissues not directly measured (e.g., gut) and for extrapolation of mouse tissue partition to human tissues. It should be noted, however, that a previous study (Fiserova-Bergerova, 1975) obtained similar tissue/air partition coefficients for MC in rat, dog, monkey, and human. For rats and humans, tissue/air partition coefficients were typically within 20%. Blood/air partition coefficients, on the other hand, varied considerably, with the human value being roughly half of that for rats — consistent with the relationship between mouse and human blood/air partition coefficients in the present study.

TABLE 5.4-1. PARTITION COEFFICIENTS FOR METHYLENE CHLORIDE

	Tissue/Air Partition	
Tissue	**Mean**	**Standard Deviation**
Mouse		
Blood (young animal)	21.8	2.6
Blood (older animal)	24.3	1.5
Liver	38.7	6.8
Kidney	11.4	1.9
Lung	10.0	2.9
Brain	9.7	1.4
Muscle	9.5	1.9
Perirenal fat	97.5	8.5
Mesenteric fat	62.0	29.0
Inguinal fat	112.0	15.0
Human		
Blood	12.9	1.2

Closed-Chamber Studies — Methylene Chloride

Figure 5.4-2 shows the results of six closed-chamber runs with MC. The model predictions shown are those for the metabolic parameters used in the new Monte Carlo analysis. The parameter values were not identified solely on the basis of these closed-chamber data, however. Figures 5.4-3 and -4, which were produced with the SimuSolv "MAP" command, show the joint confidence regions for estimating Vmaxc and Kfc from these data assuming two different values of Km. The numbers associated with the curves represent

the percent confidence that the optimal values of both parameters lie within that curve. It can be determined from these figures that the three metabolic parameters are highly colinear. The elliptical shape of the joint confidence regions in the two figures reflects the fact that Vmaxc and Kfc are negatively correlated. Moreover, the fact that the Vmaxc-Kfc confidence regions for two plausible values of Km (1 and 3.6, respectively) do not overlap demonstrates the significant interaction of Km with the other two parameters. The impact of this multiparameter colinearity is that the individual parameters cannot be reliably estimated simultaneously. In order to obtain the parameter values shown, additional experiments were performed in which the oxidative pathway was inhibited by pretreatment with TRANS (Figure 5.4-5). End-exposure blood HbCO measurements verified that oxidative metabolism had been effectively inhibited. A closed-chamber version of the dihalomethane model (Andersen et al., 1991) was used to calculate the maximum Vmaxc consistent with the measured HbCO levels. In every case, Vmaxc was calculated to be less than 0.25. Using this upper bound value for Vmaxc during TRANS inhibition, it was then possible to obtain an estimate of the lower bound (worst case, from a risk assessment standpoint) value of the parameter for the nonoxidative metabolism, Kfc, by fitting the observed total uptake in Figure 5.4-5. Figure 5.4-6 again shows a joint confidence region based on the data in Figure 5.4-2, but with Kfc fixed. As can be seen, once Kfc has been identified, the joint confidence region for the other two parameters is quite small.

Closed-Chamber Studies — Deuterated Methylene Chloride

The ideal way to determine the dose-dependent split of metabolism between two pathways is to measure the production of a metabolite associated uniquely with one pathway (Gargas and Andersen, 1982; Gargas et al., 1986b). The production of CO by the oxidative pathway provides such an opportunity, but it is first necessary to determine the relative yield of CO (moles of CO produced per mole of MC metabolized). One way to assess the relative contribution of the two competing metabolic pathways is to make use of the anticipated "isotope effect" of substituting deuterium for the two hydrogens in MC. The metabolic parameters in the MC model of Green et al. (1988) were established in this manner. However, the interpretation of the isotope effect is problematic due to the potential for effects at more than one point in the metabolic process. The assumption underlying the use of the isotope effect in the model of Green et al. was that the substitution of deuterium for hydrogen would decrease Vmaxc for the oxidative metabolism, but would not effect the stoichiometric yield of CO from the metabolism of the parent chemical. A part of our study was devoted to investigating the isotope effect for MC. Figure 5.4-7 shows the results of our closed-chamber analysis of deuterated MC. Contrary to the previously stated assumption concerning the nature of the isotope effect, it was not possible to fit these data with the same Km as MC. In fact, the data were much better simulated by making the opposite assumption: holding Vmaxc constant and increasing Km, as shown in Figure 5.4-7. In addition, a lower stoichiometric yield of CO was observed for metabolism of DMC than for MC. We are still in the process of investigating the implications of these results, but it is clear that any parameter estimation studies that have been interpreted on the basis of the previously held expectation for the isotope effect will have to be reevaluated in light of these new data.

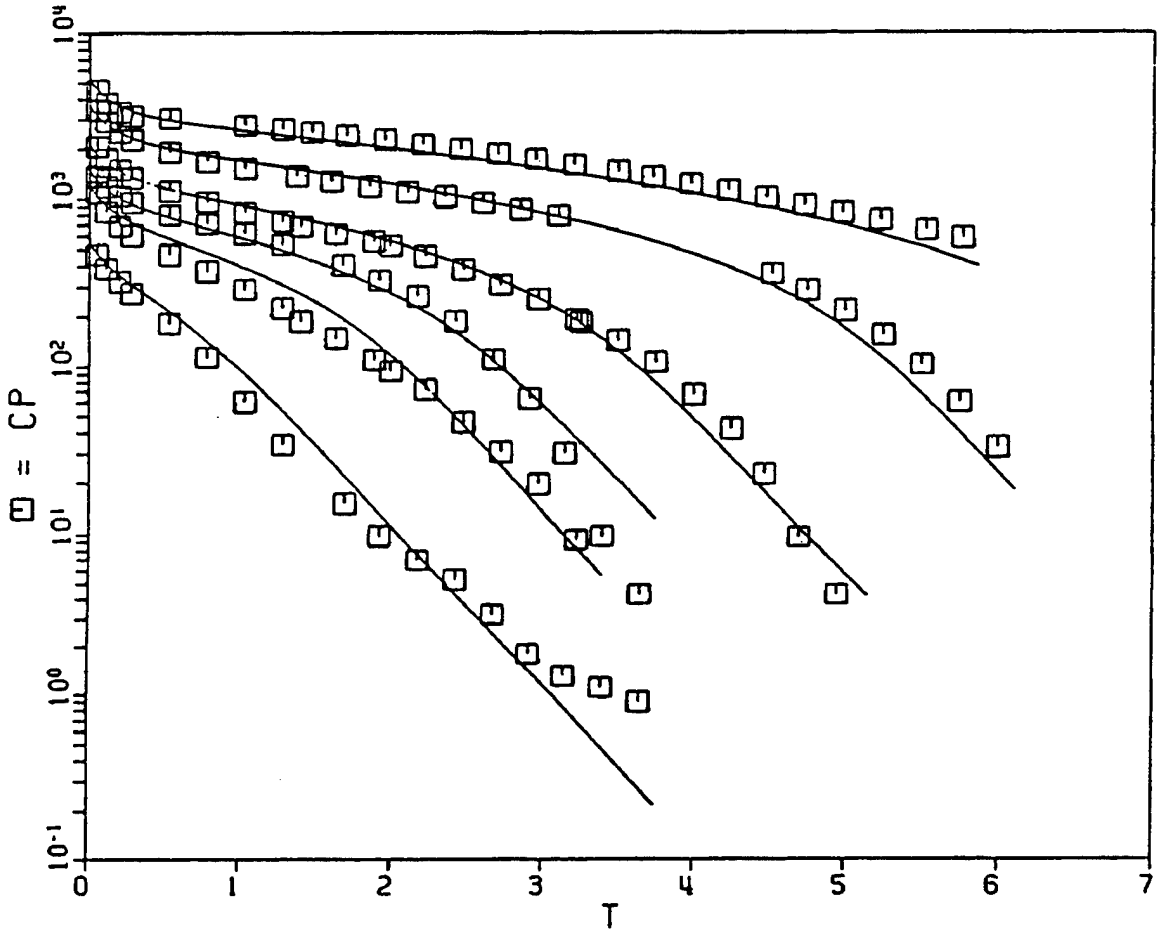


Figure 5.4-2. Closed-Chamber Results for Exposure of Female B6C3F$_1$ Mice to Methylene Chloride.

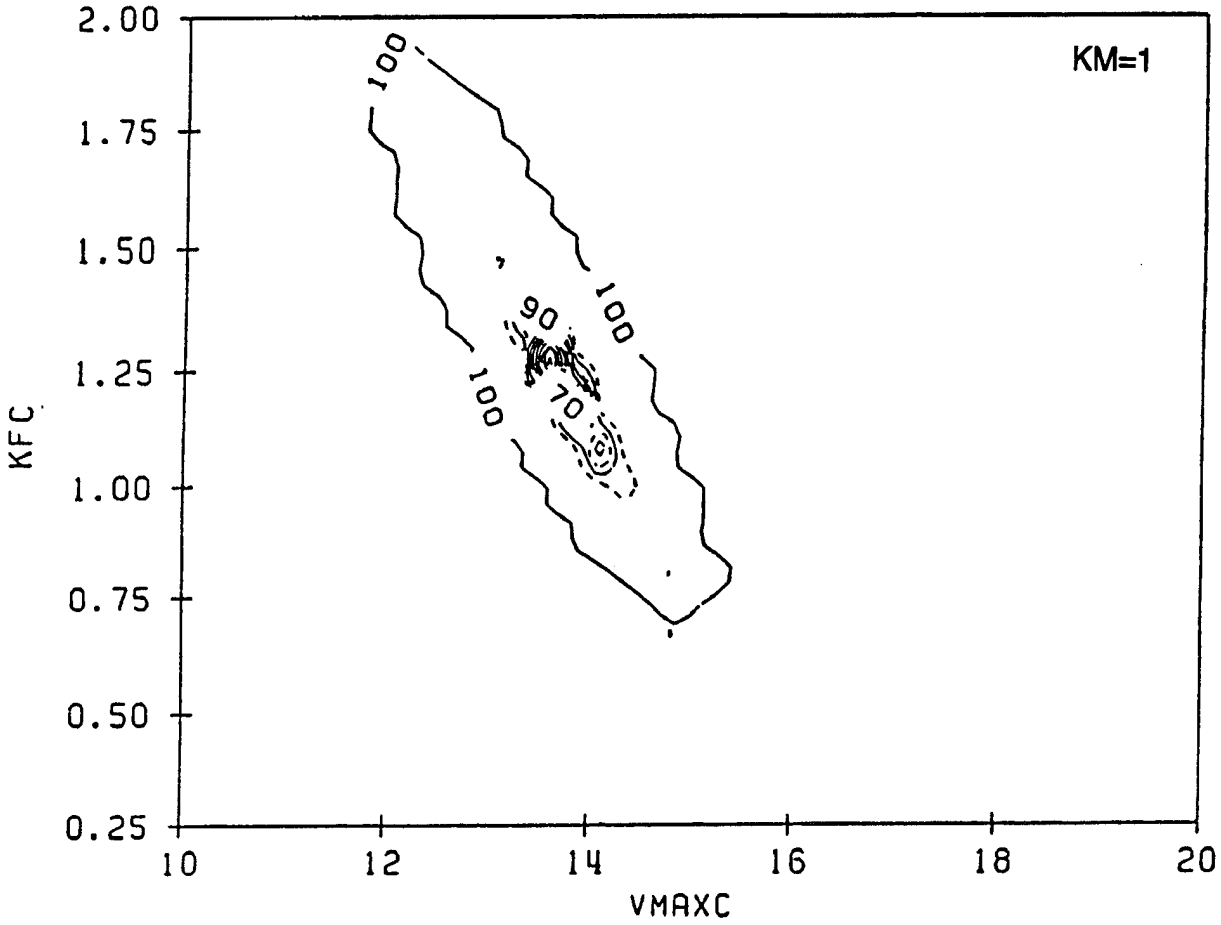


Figure 5.4-3. Confidence Region for $Vmax_c$ and Kfc Given That Km=1.

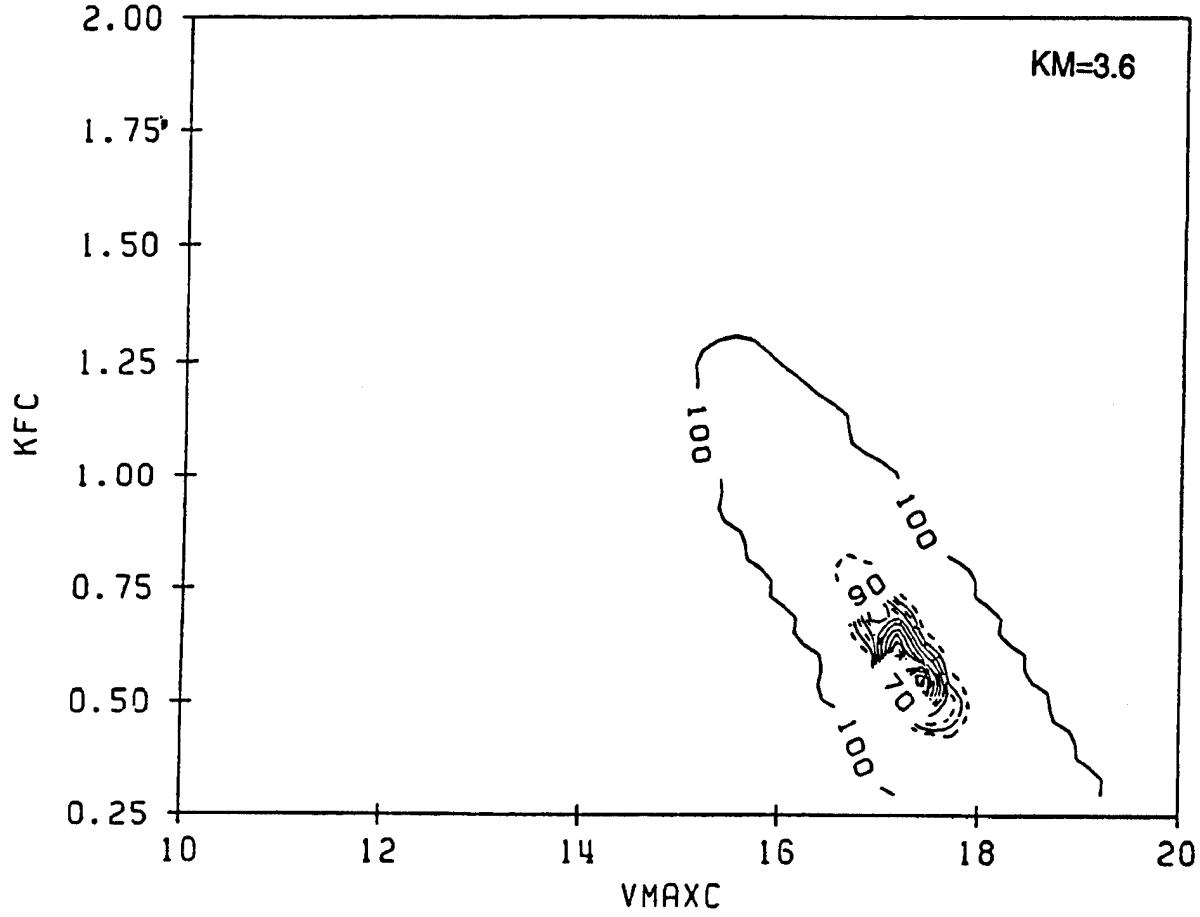


Figure 5.4-4. Confidence Region for $Vmax_c$ and Kfc Given that Km=3.6.

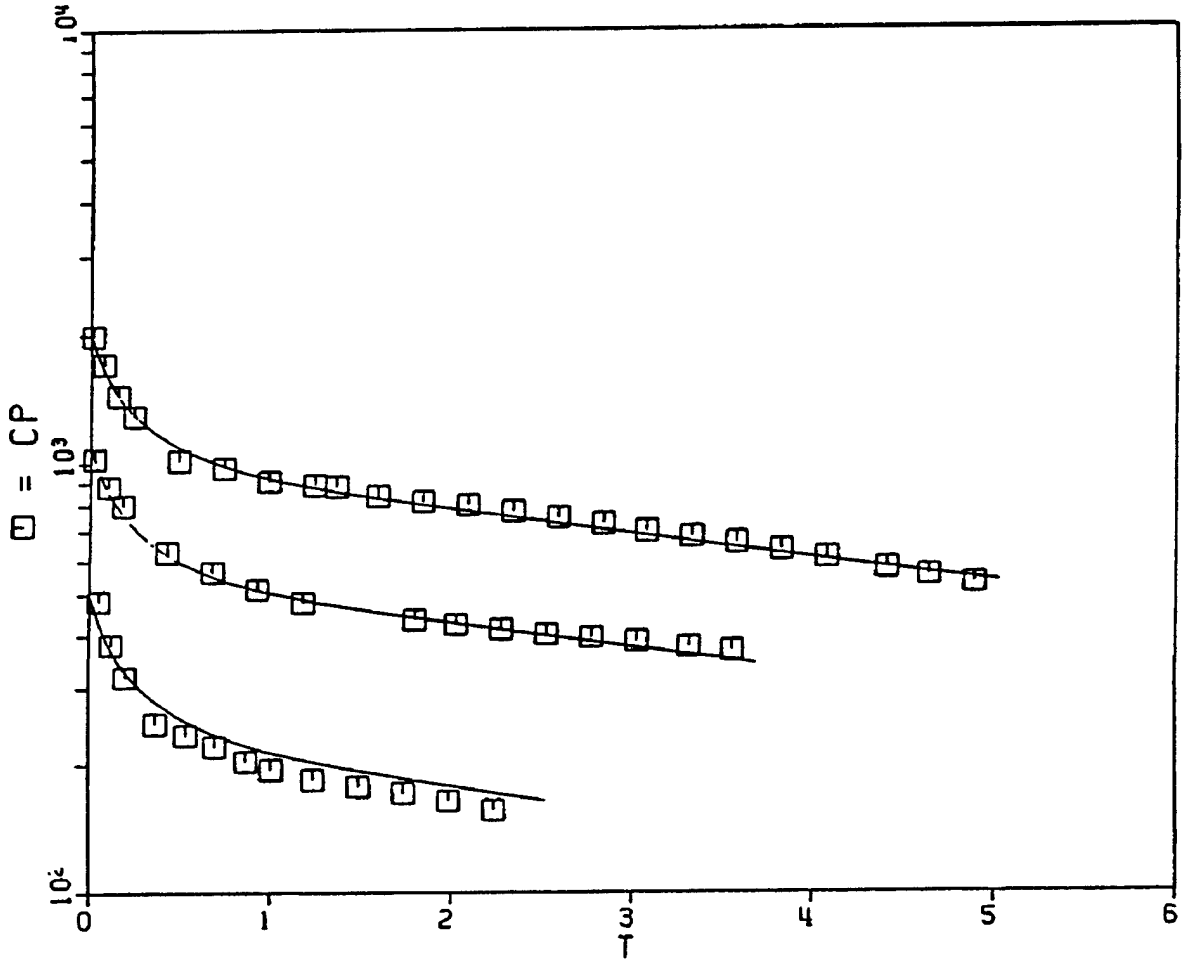


Figure 5.4-5. Closed Chamber Results for Exposure of Female B6C3F$_1$ Mice to Methylene Chloride following Exposure to *trans*-1,2-Dichloroethylene.

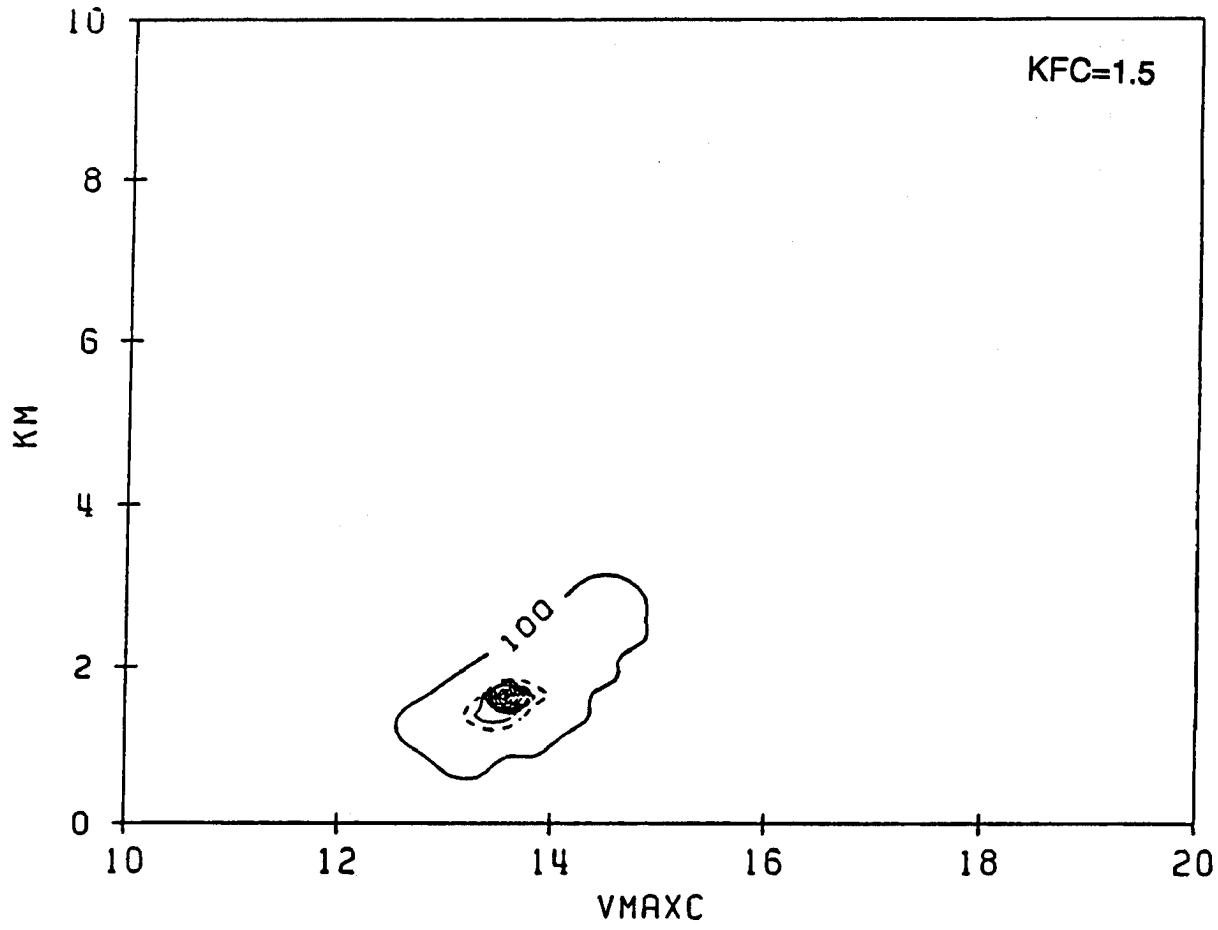


Figure 5.4-6. Confidence Region for Vmaxc and Km Given That Kfc=1.5.

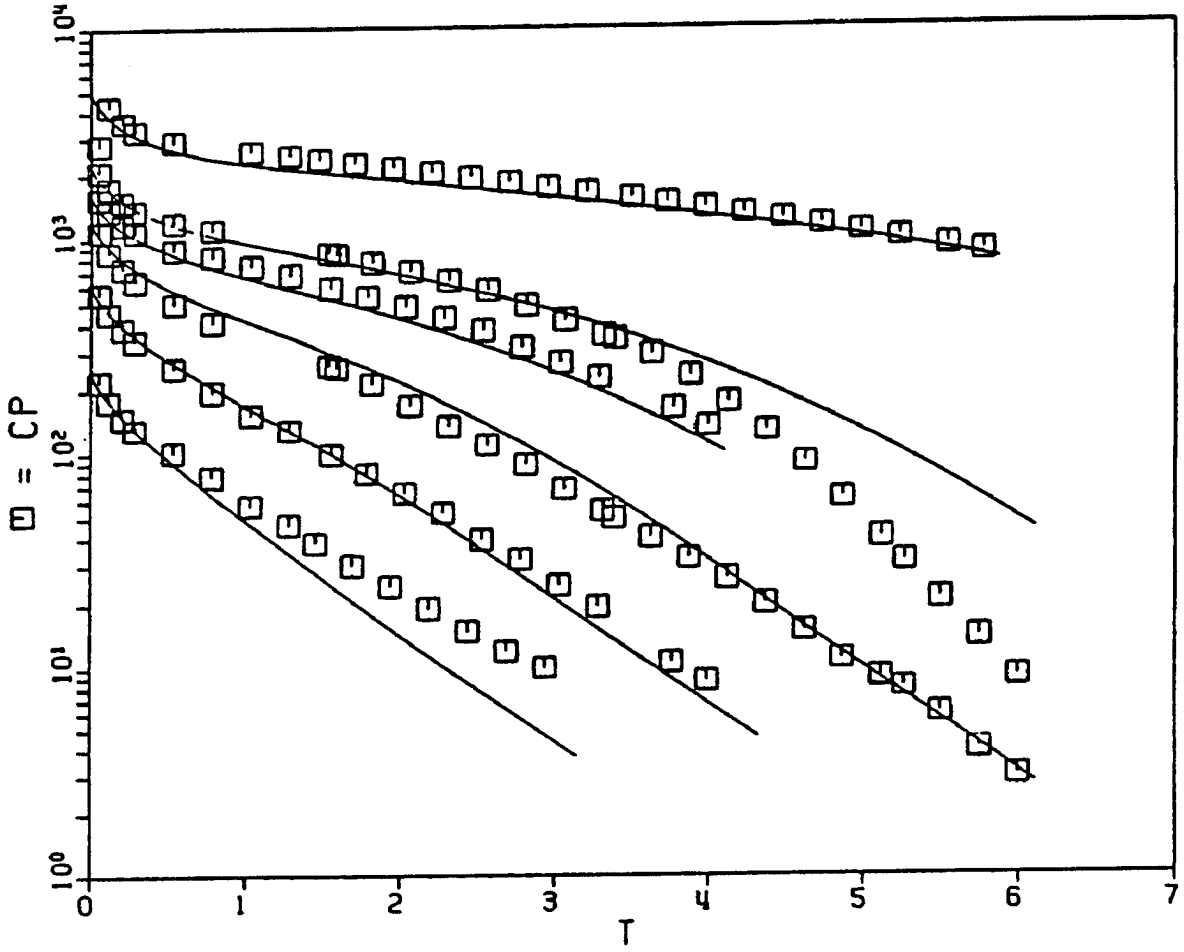


Figure 5.4-7. Closed-Chamber Results for Exposure of Female B6C3F$_1$ Mice to Deiterated Methylene Chloride.

Monte Carlo Analysis

The newly derived parameters were again used in a Monte Carlo analysis as described in our previous submissions to characterize the range of risks consistent with a pharmacokinetic approach. On the basis of the earlier analysis, which compared two alternative model formulations, a single model was selected to be used for this analysis (Appendix A). The results of the analysis are shown in Figure 5.4-8, which displays the estimated extra lifetime risk of lung cancer from exposure to MC 8 h/day, 5 days/week, for 45 years at concentrations ranging from 1 to 500 ppm. The uppermost two lines represent the OSHA risk estimates, which are based on a dose measure of milligrams per kilogram per day (calculated as the product of the ventilation rate, exposure duration, and exposure concentration), and the maximum likelihood estimate or 95% upper confidence limit for the multistage model. The four lower lines embrace the range of PBPK model-estimated risks considering the impact of uncertainty in the PBPK model parameters.

Figure 5.4-9 compares the risks estimated using a number of different dose surrogates, as before. It should be noted that of the six dose surrogates shown in Figure 5.4-9, only GST metabolism rests on sound scientific evidence. As pointed out by Dr. Richard Reitz during the OSHA hearings, the use of the MFO pathway as the dose surrogate can be ruled out on the basis of the negative MC drinking water bioassay because the amount of MFO metabolism in that study was on the same order of magnitude as in the positive inhalation bioassay. The same comparison also rules out the use of total lung metabolism (TmetP). Because the MFO pathway completely dominates lung metabolism at the concentrations achieved in both bioassays, TmetP is also at roughly the same order of magnitude in both the positive and the negative study. All of the other potential dose metrics represent measures of parent chemical exposure. But in his testimony, Dr. Robert Maronpot provided evidence from National Institute of Environmental Health Sciences studies that clearly indicates that MC acts as a classic initiator in the lung, and that essentially rules out secondary mechanisms, such as cell proliferation or cytotoxicity, by which the parent chemical might possess tumorigenic activity. Thus we are left with only the GST dose surrogate, a dose surrogate that correlates well with all available bioassay results, representing a mutagenic pathway with known reactive intermediates and evidence of DNA adduct formation. The scientific evidence is compelling that the GST pathway dose surrogate should be used to estimate human risk. The data described in this report solidify the interpretation of MC pharmacokinetics underlying the model of Andersen et al. Uncertainty regarding the model parameters can be dealt with conservatively using the approach described in this report. Based on these considerations, we strongly believe OSHA should join EPA in adopting the pharmacokinetic risk assessment methodology for MC.

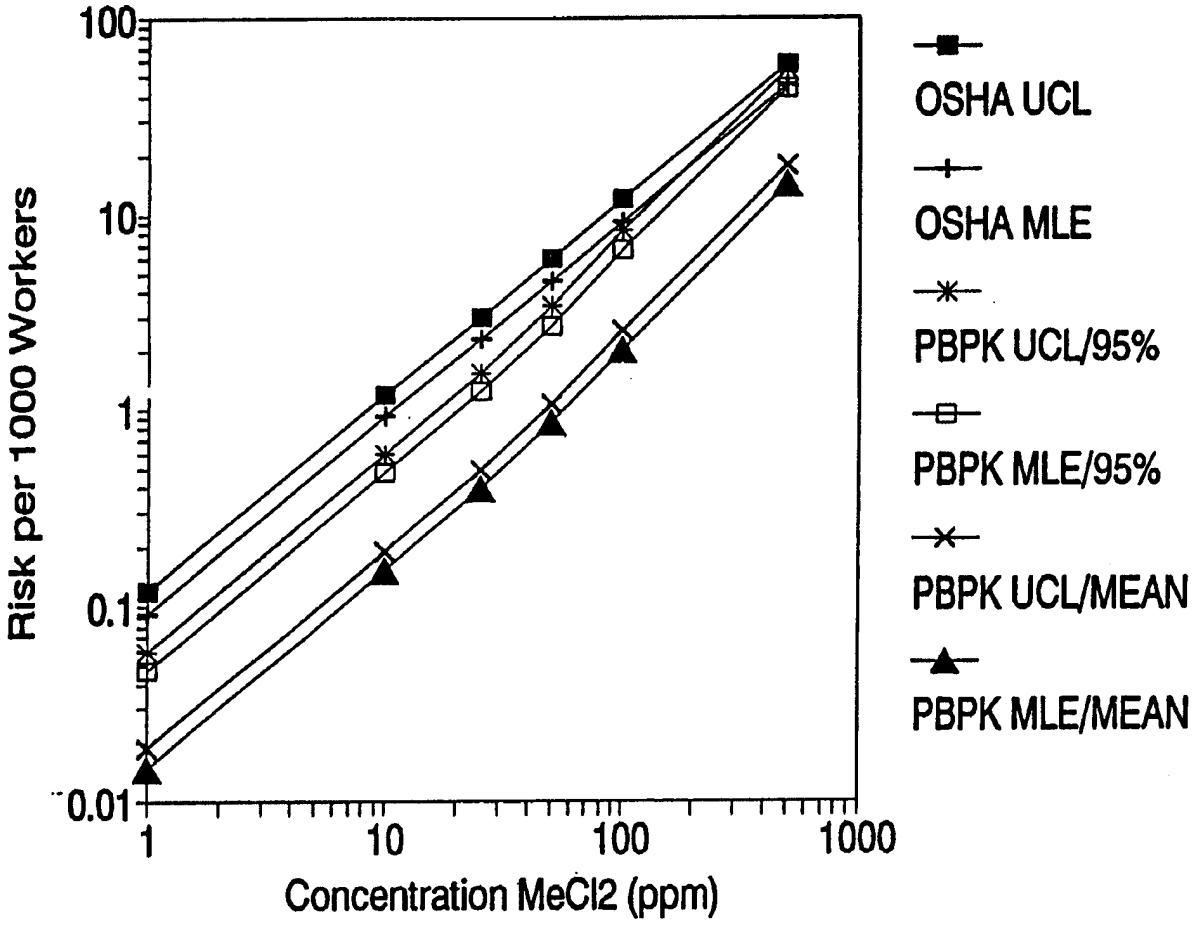


Figure 5.4-8. Comparison of Occupational Sssafety and Health Administration and Physiologically Based Pharmacokinetic (PBPK)-Based Risk Estimates for 45-Year Occupational Exposure to Methylene Chloride Based on Total Female Mouse Lung Tumors. UCL=95% upper confidence limit on risk, MLE=maximum likelihood estimate, UCL/95%=95th percentile of distribution of UCLs produced by variation of PBPK parameters independently in the mouse and the human, and MLE/MEAN=mean of distribution of MLEs produced by variation of PBPK parameters.

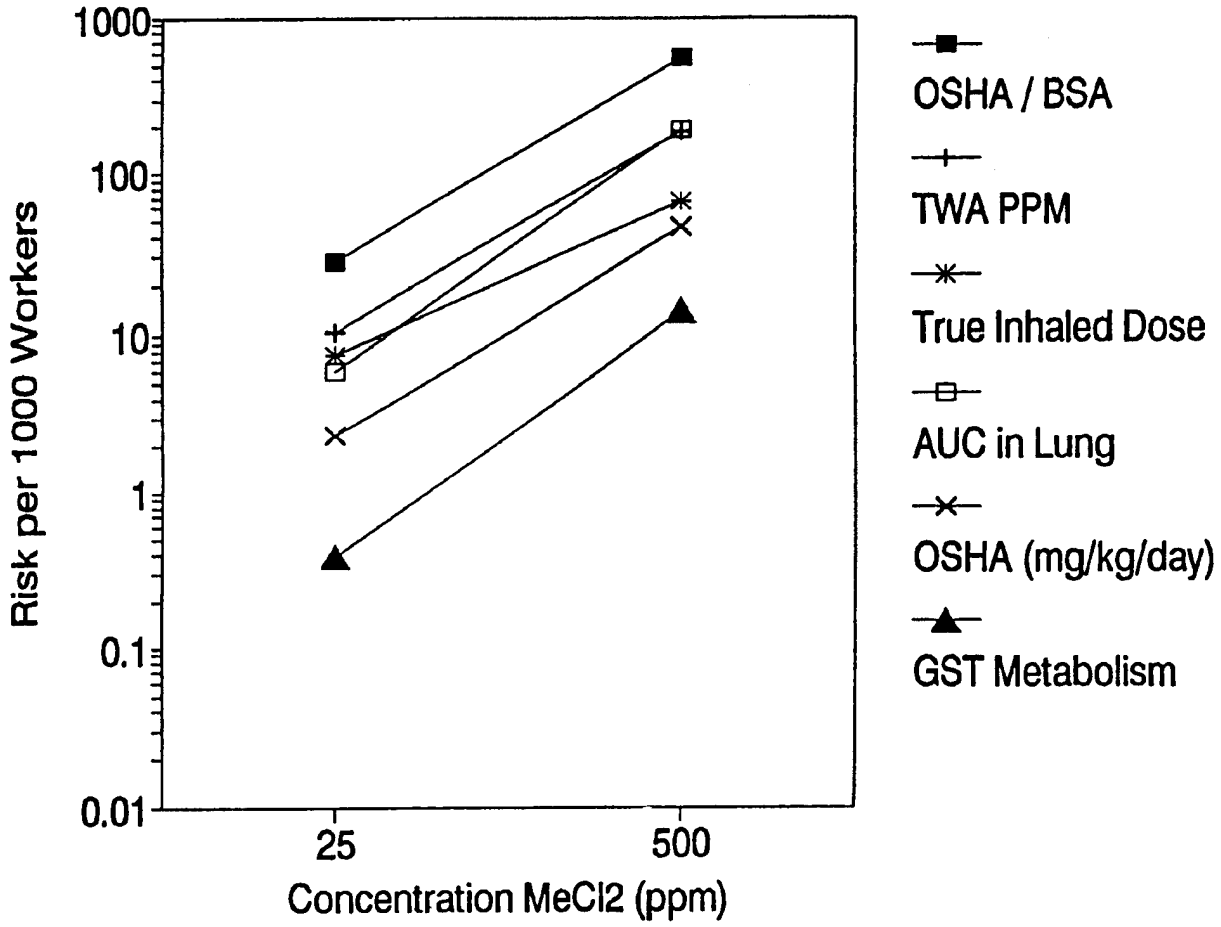


Figure 5.4-9. Comparison of Risk Estimates with Various Potential Dose Surrogates, Based on Total Female Mouse Lung Tumors. BSA=body surface area cross-species adjustment, TWA PPM=time-weighted-average parts per million, AUC=area under the curve, and GST=glutathione-*S*-transferase.

REFERENCES

Andersen, M.E., H.J. Clewell, III, M.L. Gargas, F.A. Smith, and R.H. Reitz. 1987. Physiologically based pharmacokinetics and the risk assessment process for methylene chloride. *Toxicol. Appl. Pharmacol.* 87:185-205.

Andersen, M.E., H.J. Clewell III, M.L. Gargas, M.J. MacNaughton, R.H. Reitz, R. Nolan, and M. McKenna. 1991. Physiologically based pharmacokinetic modeling with dichloromethane, it's metabolite carbon monoxide, and blood carboxyhemoglobin in rats and humans. *Toxicol. Appl. Pharmacol.* 108:14-27.

Fiserova-Bergerova, V. 1975. Biological-mathematical modeling of chronic toxicity. AMRL-TR-75-5. Wright-Patterson Air Force Base, OH: Aerospace Medical Research Laboratory.

Gargas, M.L. and M.E. Andersen. 1982. Metabolism of inhaled brominated hydrocarbons: Validation of gas uptake results by determination of a stable metabolite. *Toxicol. Appl. Pharmacol.* 66:55-68.

Gargas, M.L., M.E. Andersen, and H.J. Clewell III. 1986a. A physiologically based simulation approach for determining metabolic constants from gas uptake data. *Toxicol. Appl. Pharmacol.* 86:341-352.

Gargas, M.L., M.E. Andersen, and H.J. Clewell III. 1986b. Metabolism of inhaled dihalomethanes: Differentiation of kinetic constants for two independent pathways. *Toxicol. Appl. Pharmacol.* 87:211.

Gargas, M.L., R.J. Burgess, D.E. Voisard, G.H. Cason, and M.E. Andersen. 1989. Partition coefficients of low-molecular-weight volatile chemicals in various liquids and tissues. *Toxicol. Appl. Pharmacol.* 98:87-99.

Green, T., W.M. Provan, P.H. Dugard, and S.K. Cook. 1988. Methylene chloride (dichloromethane): Human risk assessment using experimental animal data. ECETOC Technical Report No. 32. Brussels, Belgium.

Rodkey, F.L., T.A. Hill, L.L. Pitts, and R.F. Robertson. 1979. Spectrophotometric measurement of carboxyhemoglobin and methemoglobin in blood. *Clin. Chem.* 25(8):1388-1393.

APPENDIX A

PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL DEFINITION AND RISK CALCULATIONS

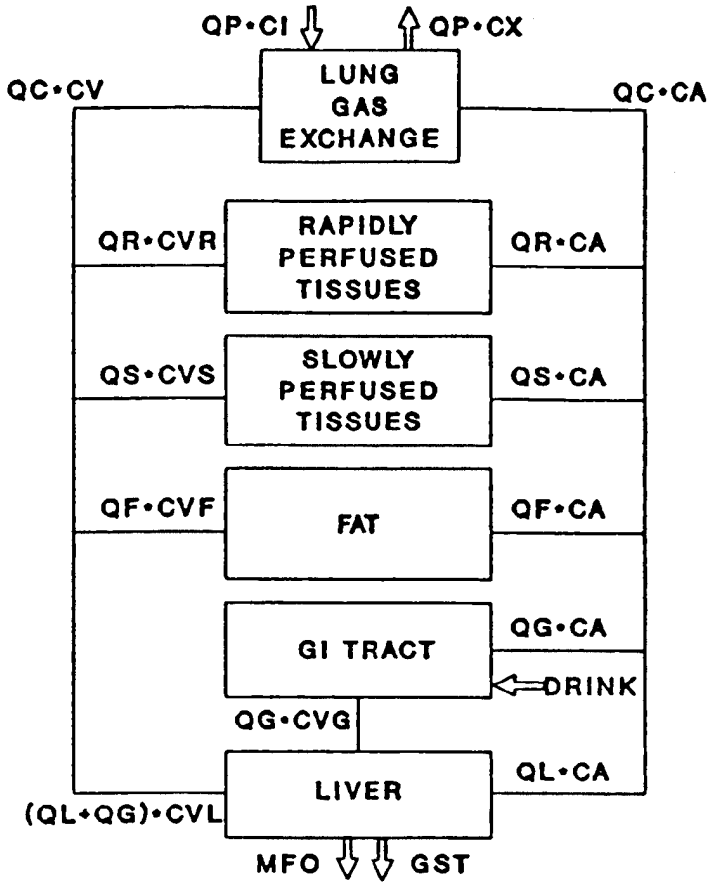


Figure 5.4A-1. Diagram of Physiologically Based Pharmacokinetic Model for Methylene Chloride Risk Assessment.

TABLE 5.4A-1. MODEL PARAMETERS AND DOSE SURROGATES

UNSCALED PARAMETERS:		Value		CV (%)	
		Mouse	Human	Mouse	Human
BW	Body Weight (kg)	0.0345	70	11	30
QPC	Alveolar Ventilation (L/h, 1 kg animal)	29.0	35	58	15.8
QCC	Cardiac Output (L/h, 1 kg animal)	16.5	18	8.5	9.1
Tissue Blood Flows (Fraction of Cardiac Output):					
QGC	Flow to Gastrointestinal (GI) Tract	0.165	0.195	25	10
QLC	Flow to Liver	0.035	0.07	96	35
QFC	Flow to Fat	0.03	0.05	60	30
QSC	Flow to Slowly Perfused Tissues	0.25	0.24	40	15
QRC	Flow to Rapidly Perfused Tissues	0.52	0.445	50	20
Tissue Volumes (Fraction of Body Weight):					
VGC	Volume of GI Tract	0.031	0.045	30	10
VLC	Volume of Liver	0.046	0.023	6	5
VFC	Volume of Fat	0.100	0.16	30	30
VSC	Volume of Slowly Perfused Tissues	0.513	0.48	30	30
VRC	Volume of Rapidly Perfused Tissues	0.041	0.033	30	10
VLUC	Volume of Lung Tissue	0.008	0.006	30	10
Partition Coefficients:					
PB	Blood/Air	23.0	12.9	15	10
PG	GI Tract/Blood	0.52	0.93	30	30
PL	Liver/Blood	1.6	2.9	20	20
PF	Fat/Blood	5.1	9.1	30	30
PS	Slowly Perfused Tissue/Blood	0.44	0.78	20	20
PR	Richly Perfused Tissue/Blood	0.52	0.93	20	20
PLU	Lung/Blood	0.46	0.82	30	30
Metabolic Parameters:					
VMAXC	Maximum Velocity of Saturable Pathway (mg/h, 1 kg animal)	13.4	5.0	20	30
KM	Affinity of Saturable Pathway (mg/L)	1.35	0.4	30	50
KFC	Rate Constant for 1st Order Pathway (/h, 1 kg animal)	1.5	1.5	30	50
A1	Vmaxc(Lung)/Vmaxc(Liver)	0.41	0.015	50	70
A2	KFC(Lung)/KFC(Liver)	0.28	0.18	50	70

TABLE 5.4A-1. MODEL PARAMETERS AND DOSE SURROGATES (CONTINUED)

SCALED PARAMETERS:

VG = VGC*BW
VL = VLC*BW
VF = VFC*BW
VS = VSC*BW
VR = VRC*BW
VLU = VLUC*BW
VMAX = VMAXC*BW**0.75
KF = KFC/BW**0.25

QP = QPC*BW**0.75
QC = QCC*BW**0.75
QG = QGC*QC
QL = QLC*QC
QF = QFC*QC
QS = QSC*QC
QR = QRC*QC

Note: When the input parameters are subject to modification by a Monte Carlo or sensitivity analysis, it is generally necessary to recompute the total blood flow in order to maintain mass balance:

QCI = QCC*BW**0.75
QG = QGC*QCI
QL = QLC*QCI
QF = QFC*QCI
QS = QSC*QCI
QR = QRC*QCI
QC = QG + QL + QF + QS + QR

(where QCC, QGC, QLC, QFC, QSC, and QRC are subject to modification)

The exception is when sampling is performed in a way that constrains the sum of the blood flow fractions to equal 1, as with the Dirichlet distribution.

DOSE SURROGATES:

AUCL	Area under the curve of liver concentration of $MeCl_2$
Risk1L	Amount metabolized by the liver linear pathway/VL
Risk2L	Amount metabolized by the liver saturable pathway/VL
TmetL	Total amount metabolized in liver/VL
AUCLU	Area under the curve of lung concentration of $MeCl_2$
Risk1LU	Amount metabolized by the lung linear pathway/VLU
Risk2LU	Amount metabolized by the lung saturable pathway/VLU
TmetP	Total amount metabolized in lung/VLU
Tdose	Total amount inhaled during exposure = AUC of QP*(CI-CX) for exposure period only
Tmet	Total amount metabolized (liver plus lung)/BW

TABLE 5.4A-2. COMPARISON OF OCCUPATIONAL SAFETY AND HEALTH ADMINISTRATION (OSHA) AND PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK)-BASED RISK ESTIMATES PER 1000 WORKERS FOR 45-YEAR OCCUPATIONAL EXPOSURE TO METHYLENE CHLORIDE (Based on National Toxicology Program – Female Mice, Alveolar/Bronchiolar Adenoma or Carcinoma).

Methylene Chloride Concentration (ppm)	OSHA[a]		PBPK[b] (mean)		PBPKb (95%)	
	MLE[d]	95% UCL[d]	MLE[d]	95% UCL[d]	MLE[d]	95% UCL[d]
1	0.0930	0.119	0.0148	0.0185	0.0472	0.0591
10	0.930	1.19	0.151	0.189	0.480	0.601
25	2.32	2.97	0.392	0.491	1.24	1.56
50	4.64	5.92	0.852	1.06	2.71	3.40
100	9.26	11.8	2.03	2.53	6.61	8.28
500	45.5	57.7	14.1	17.6	44.2	55.0

[a] OSHA Inhaled Dose Estimate (mg/kg/day)

[b] Methylene Chloride PBPK Model Estimate (GST Pathway Dose Surrogate)

[c] Mean and 95th percentile of 500 Monte Carlo simulations of PBPK model parameters

[d] Maximum likelihood estimate (MLE) and 95% upper confidence limit (UCL) of extra lifetime risk as estimated with a one-stage model

TABLE 5.4A-3. COMPARISON OF RISK ESTIMATES PER 1000 WORKERS FOR 45-YEAR OCCUPATIONAL EXPOSURE TO METHYLENE CHLORIDE FOR VARIOUS DOSE SURROGATES (Based on National Toxicology Program – Female Mice, Alveolar/Bronchiolar Adenoma or Carcinoma).[a]

a: 25 ppm Methylene Chloride

	MLE	Ratio	UCL	UCL/95%
mg/kg/day - BSA	28.6	12.3	36.6	--
PPM - TWA	10.4	4.5	12.8	--
Inhaled Dose - PBPK	7.54	3.25	9.39	13.1
AUC (Lung) - PBPK	5.97	2.6	7.46	15.3
Risk1P (MFO) - PBPK	2.96	1.3	3.68	9.73
mg/kg/day - OSHA	**2.32**	**1**	**2.97**	--
TmetP - PBPK	1.83	0.9	2.28	6.12
Risk2P (GST) - PBPK	0.39	0.2	0.49	1.56

b: 500 ppm Methylene Chloride

	MLE	Ratio	UCL	UCL/95%
mg/kg/day - BSA	**561.5**	**12.3**	**712**	--
PPM - TWA	**188**	**4.1**	**227**	--
Inhaled Dose - PBPK	66.3	1.5	81.9	114
AUC (Lung) - PBPK	190	4.2	231	416
Risk1P (MFO) - PBPK	9.05	0.2	11.2	30.1
mg/kg/day - OSHA	**45.5**	**1**	**57.7**	--
TmetP - PBPK	9.33	0.2	11.6	28.2
Risk2P (GST) - PBPK	14.1	0.3	17.6	55.0

[a] MLE = maximum likelihood estimate, UCL = upper confidence limit; BSA = body surface area cross-species adjustment, TWA = time-weighted average, PBPK = physiologically based pharmacokinetic, AUC = area under the curve, Risk1P = risk determined from the oxidative pathway, OSHA = Occupational Safety and Health Administration, TmetP = total amount metabolized in lung divided by the ratio of volume of lung tissue to body weight, Risk2P = risk determined from the glutathione pathway, GST = glutathione-*S*-transferase.

6.1 RESEARCH ENGINEERING SPECIAL PROJECTS

H.F. Leahy, W.B. Sonntag, D.L. Courson, and W.J. Malcomb

The Toxic Hazards Research Unit (THRU) Research Engineering staff has provided technical assistance on a number of research and special projects. This report describes the devices, instruments, and systems that were designed, fabricated, and applied to support several THRU projects. In addition to performing routine maintenance of specialized research systems at the THRU, major renovations of existing systems were undertaken to address efficiency and safety of operation. Many of the systems that were developed by the Research Engineering group are described in separate sections of this report.

INHALATION EXPOSURES WITH CHLOROFLUOROCARBON REPLACEMENT COMPOUNDS

Continued assistance was provided to the THRU research teams by conducting inhalation exposures to various chlorofluorocarbon replacement compounds. Details on exposure chambers and conditions have been reported in the 1992 THRU Annual Report (Wall et al., In Press). A compartmentalized box was used when there was a need for drawing blood samples during exposures and a bell jar was used when there was no need for drawing blood samples during exposures. Analysis of the exposure atmosphere was performed using a Miran 1A equipped with either a 10-cm cell for the higher concentrations or the variable-length cell (0.75 to 20 m) for the lower concentrations. The Varian 3400 was used for the last two exposures in the bell jar. Table 6.1-1 contains information on test material, exposure concentration, and method of analysis.

An exposure to HCFC-123 was conducted in a reduced oxygen atmosphere, by addition of nitrogen to the incoming air. An oxygen probe was used for monitoring the oxygen, which was maintained at 14%. The same probe was used for the 10% vapor tests of perfluorohexane and Halon 1211 when oxygen concentrations of 18 to 19% were recorded. The exhaust vapors of these studies were passed through an activated charcoal filter before discharge.

ANALYSIS OF DRINKING WATER SOLUTIONS OF LIQUID PROPELLANT 1846

Concern for the stability of Liquid Propellant 1846 (XM46) in water led to testing the pH as well as the nitrate ion concentration of stored and fresh samples taken from the various batches of drinking water prepared for the modified Screening Information Data Set test. XM46 is composed of 61% hydroxylammonium nitrate (HAN), 19% triethanolaminonitrate (TEAN), 0.1% nitric acid, and 20% water.

TABLE 6.1-1. LIST OF EXPOSURES PERFORMED FOR STUDY NO. F08

Test Material	Concentration %	Concentration SD	Time (h)	Chamber[a]	Analysis Method	Oxygen (%)
HCFC-123	2.51	0.04	2	Jar	Miran	14
HCFC-123	1.02	0.04	4	Box	Miran	Normal
PFH	1.00	0.04	4	Box	Miran	Normal
PFH	10.1	0.1	4	Box	Miran	18-19
Halon-1211	1.05	0.02	4	Box	Miran	Normal
Halon-1211	10.21	0.12	4	Box	Miran	18-19
CDFE	1.01	0.07	4	Box	Miran	Normal
CDFE	1.01	0.09	4	Box	Miran	Normal
CDFE	0.010	0.000	4	Box	Miran	Normal
CDFE	0.010	0.000	4	Box	Miran	Normal
Control	—	—	4	Box	Miran	Normal
HCFC-123	1.03	0.05	2	Box	Miran	Normal
HCFC-123	0.102	0.005	6	Box	Miran	Normal
HCFC-123	0.103	0.003	4	Box	Miran	Normal
HCFC-123	0.102	0.003	4	Box	Miran	Normal
HCFC-123	0.103	0.001	4	Box	Miran	Normal
HCFC-123	0.104	0.001	4	Box	Miran	Normal
Halothane	0.101	0.006	4	Box	Miran	Normal
Halothane	0.101	0.007	4	Box	Miran	Normal

[a]Jar = 30-L bell jar, Box = 11-L Plexiglas compartmentalized chamber.

A Model 25 pH/ion meter (Fisher Scientific, Pittsburgh, PA) using a pH probe (Fisher Cat.# 13-620-285) with accompanying temperature probe (Fisher Cat.# 13-620-16) was used for pH analysis, and an Orion Model 93-07 nitrate ion probe (Orion Instruments, Boston, MA) with a companion reference electrode (Fisher, Double Junction Electrode, ISE Cat.# 13620-47) was used for nitrate analysis.

The reference buffers were pH 4.00 and 10.00 from Aldrich (Aldrich Chemical Co, Milwaukee, WI) and pH 7.00 from Fisher Scientific. The 100- and 1000-ppm nitrate ion standards were obtained from Orion, and the 1- and 10-ppm standards were made by dilution of the 100-ppm standard with laboratory-supplied reverse osmosis (RO) water.

The 2.0 g/L dilution of XM46 was performed while monitoring the pH. The results of this test demonstrated that the initial pH approximated that expected from the 0.1% nitric acid added to stabilize XM46. This occurred immediately upon the addition of XM46 to the water. Little change was observed for short-term storage and, even after 2 months, the change in the highest concentration solution was from a pH of 4.3 to 3.1 (Table 6.1-2). The drinking water test solutions were remade every 5 to 7 days, as needed.

TABLE 6.1-2. SUMMARY OF pH TESTING OF XM46 DOSING SOLUTION IN DRINKING WATER STUDY

		pH			
Date Prepared	Date Analyzed	Reverse Osmosis Water	0.2 g/L	1.0 g/L	2.0 g/L
13 JUL	16 SEP	6.91	3.47	3.21	3.15
15 JUL	16 SEP	6.86	3.53	3.31	3.24
20 JUL	16 SEP	6.71	3.57	3.31	3.24
22 JUL	16 SEP	6.75	3.59	3.38	3.29
28 JUL	16 SEP	6.65	3.66	3.44	3.34
05 AUG	16 SEP	6.67	3.69	3.45	3.38
12 AUG	16 SEP	6.64	3.74	3.51	3.41
19 AUG	16 SEP	6.74	3.82	3.59	3.50
26 AUG	16 SEP	6.84	3.87	3.63	3.55
02 SEP	16 SEP	6.56	4.02	3.79	3.72
08 SEP	16 SEP	6.70	4.20	4.01	3.88
09 SEP	16 SEP	6.55	4.21	3.99	3.88
14 SEP	16 SEP	6.62	4.66	4.33	4.16
22 SEP (Rats)	24 SEP	6.70	4.56	3.97	3.83
24 SEP	24 SEP	6.72	5.30	—	—
28 SEP	28 SEP	6.63	5.27	4.75	4.36
01 OCT	01 OCT	6.57	5.28	4.53	4.28
07 OCT	08 OCT	6.74	4.94	4.49	4.27

The nitrate ion probe measures nitrate ion activity, (i.e., only the ionized form). Both HAN and TEAN, at the dilutions used in this study, ionized immediately and completely. The molar concentrations of the solutions were in the range expected from the combined concentrations. It also appeared that the solutions did not change with respect to the nitrate ion even during long-term storage (Table 6.1-3). Nitrate standards were run with each batch of test samples. The slopes of all curves were between -56 to -60 mV for 1 log difference in concentration and were linear across the range of samples and standards.

TABLE 6.1-3. SUMMARY OF NITRATE ANALYSIS (μmol/mL) OF XM46 SOLUTIONS IN DRINKING WATER SAMPLES

		Nitrate			
Date Prepared	Date Analyzed	Reverse Osmosis Water	0.2 g/L	1.0 g/L	2.0 g/L
13 JUL	21 SEP	0.05	2.58	9.16	21.13
15 JUL	21 SEP	0.05	1.88	8.42	13.86
20 JUL	21 SEP	0.05	1.72	7.76	13.70
22 JUL	21 SEP	0.05	1.62	7.35	13.81
28 JUL	21 SEP	0.05	1.74	7.64	13.81
05 AUG	21 SEP	0.05	1.64	7.52	13.70
12 AUG	21 SEP	0.05	1.48	6.99	13.54
19 AUG	21 SEP	0.05	1.66	7.29	13.49
26 AUG	21 SEP	0.06	1.55	7.38	13.97
02 SEP	21 SEP	0.06	1.55	7.15	13.33
08 SEP	21 SEP	0.05	1.78	6.39	13.81
09 SEP	21 SEP	0.05	1.51	7.04	13.81
14 SEP	21 SEP	0.05	1.61	7.32	13.39
20 SEP	21 SEP	0.05	1.50	6.88	12.88
24 SEEP	24 SEP	0.06	1.43	—	—
28 SEP	28 SEP	0.06	1.42	7.84	14.00
01 OCT	01 OCT	0.06	1.46	8.64	15.70
07 OCT	08 OCT	0.06	1.55	7.12	14.70

GENERATION AND ANALYSIS OF TOLUENE VAPORS IN THRU AMBIENT CHAMBERS

Exposure Engineering and Chemistry Support personnel assisted in the operation of four of the THRU ambient chambers for this combined Air Force and Ohio State University research project. A 4-h exposure period was provided daily for 2 weeks prior to the introduction of the test compound in order to stabilize and record the individual menstrual cycles under the test housing conditions. Exposures continued for 26 days. The test compound, toluene, was maintained at the three test levels of 100, 500, and 1000 ppm along with a control group.

The chambers were operated at 6.6 cfm, providing an exchange rate of more than 12 volumes/h. The desired vapor concentrations were generated by sparging toluene in gas washing bottles. For better output control, the two higher concentration generator systems were operated in water baths maintained at 23 °C.

The chamber atmospheres were monitored using long path infrared analyzers (Miran 1A, Foxborough Inst. Corp, South Norwalk, CT). The path length and the range setting for amplification of the output signals

were chosen to operate the recorders between 50 and 75% of full scale. Due to the volume contained in the long path, the standards were made using 50-L Tedlar bags of known concentrations.

GENERATION AND ANALYSIS OF IODOTRIFLUOROMETHANE FOR NOSE-ONLY RAT EXPOSURES

The 1% and 0.5% V/V exposures of rats to iodotrifluoromethane (CF_3I) as well as controls were performed in a recently designed nose-only system. The instrument consists of two concentric pipes, vertically mounted, with the input at the top into the inner pipe and the exhaust from the bottom of the outer one. Fifty-two ports, vertically mounted in sets of four, are available for use in this system. The input air is delivered to the animals' breathing zones by short tubes extending radially from the inner pipe, and the return is exhausted through the outer pipe. The small volume of the chamber as well as the flow pattern of the air permit exposures to be performed with a minimal amount of material. This was important due to the expense of the material and for minimizing disposal problems for the exhaust air. The system was maintained at a pressure of -1 in. of water relative to the room during the exposures.

Considering the efficiency of this nose-only system, a 10-L/min flow was sufficient for the 30 animals in the three tests. The CF_3I, a liquid under pressure, was maintained at constant temperature (22 °C) with a water bath, and the vapor was metered through a Matheson 601 rotameter and discharged into the airflow in a countercurrent manner to aid good mixing. The rotameter was calibrated using an all-glass bubble flowmeter.

A Varian 3400 gas chromatograph (GC) equipped with a loop injection system was used because of the minimal sample requirement and the rapidity of response (1 injection/min, with a retention time of 0.48 min, repeated in groups of five). A 15 m×0.53 mm wide-bore fused silica capillary column, wall coated with SPB-5, was used with flame ionization detection. The column temperature was isothermal at 50 °C. The sample for the input air was taken at 25 mL/min from one of the centrally located ports. The negative pressure (-6.5 in. of water) in the loop system was operated during exposures and during calibration.

NOSE-ONLY EXPOSURES OF TRICHLOROETHYLENE TO B6C3F$_1$ MICE

A series of exposures of mice for 7 h to 600 ppm trichloroethylene (TCE) was performed to determine kinetics during and following inhalation. An important feature was to provide access to individual animals at specific time intervals during the exposure without affecting the remaining subjects. Adaption of a stainless steel nose-only exposure system (Model 70052, Lab Products, Aberdeen, MD) provided the rapid-access system needed for this study.

The nose-only system consisted of two vertical concentric stainless steel pipes, 28 in. long, with the input atmosphere entering from the top into the internal pipe and distributed to the animal holding tubes. The exhaust air was removed from the bottom of the system. There were 52 places in the system for the holding tubes, in 4 rows of 13 tubes per row. Eighteen small rat holding tubes, with a volume of 350 mL each, were used as individual holding cages for the exposure. The unused positions were closed off and the radiating delivery pipes at those positions were replaced with plugs. At specific time points, an animal was removed and the port was sealed, resulting in little effect on exposure conditions at other animal positions except a short-term, slight loss of negative pressure in the system.

The TCE ($Cl_2C=CClH$), CAS number 79-01-6, was purchased from Aldrich Chemical Co., Inc., Milwaukee, WI (Lot# 04027, F.W. 131.39). The target concentration of 600 ppm TCE was generated using a 250-mL gas washing bubbler. The TCE supply was maintained at 0 °C with a slush ice bath to provide consistent vapor pressure as well as to give a better control with the sparging airflow (about 220 mL/min). The concentrated TCE in air was delivered into the input air line in a counter flow direction to aid mixing. The total flow for the system was 10 L/min. This provided more than 500 mL/min for each chamber in use (i.e., more than one chamber volume exchange per minute). The input system and the small dead volume permitted very rapid buildup to the desired concentration, about 2 to 4 min from start-up. The push-pull air flow system was operated to maintain a slight negative pressure (-0.6 $\pm$ 0.1 in. of water) on the exhaust flow. The air passed through a charcoal filter prior to being exhausted from the building.

The concentration was monitored using a Varian 3400 (Varian Associates, Palo Alto, CA) with a loop injection system. The integrator was activated after each group of five injections, spaced 1 min apart. The 15 m$\times$0.53 mm SPB-5 column was operated isothermally at 175 °C and the chromatograph was equipped with a flame ionization detector. The built-in relay system was used for timing and the onboard integrator was used for integration of the peaks. Calibration was performed prior to the start of the exposures using Tedlar bags of known concentrations. The loop was operated at the same pressure during calibration as during exposure analysis. The GC sample was taken from a centrally located port at 25 mL/min (1/20 of the input rate to a rat holding tube). Table 6.1-4 provides a summary of the TCE exposure data.

Airflows were monitored using Matheson rotameters (Matheson Gas Products, Secaucus, NJ). The system required attention due to fluctuations in the house air supply system. Changes in pressure could unbalance the relatively small volume and tight-sealed push-pull system. When required, very slight adjustments of the exhaust flow rebalanced the system.

TABLE 6.1-4. SUMMARY OF TRICHLOROETHYLENE EXPOSURE DATA; TARGET 600 PPM FOR 7 h

	Exposure Number				
	1	2	3	4	5
Count (N)	76.0	75.0	75.0	76.0	76.0
Sum	45610.5	45296.5	45265.6	45717.9	45904.1
Mean	600.1	604.0	603.5	601.6	604.0
Standard Error of the Mean	0.9	1.0	0.7	0.6	0.7
Median	601.9	604.9	603.0	601.9	603.8
Variance	56.3	72.3	34.4	28.7	42.1
Standard Deviation	7.5	8.5	5.9	5.4	6.5
Maximum	607.7	618.6	617.8	616.2	623.7
Minimum	556.4	549.7	579.3	588.9	587.5
Range	51.3	68.9	38.5	27.2	36.2
Skewness	-3.2	-4.0	-0.2	0.2	0.1
Kurtosis	15.3	23.1	3.7	0.1	0.4

CONSTRUCTION, CALIBRATION, AND OPERATION OF A FLUIDIZED BED GENERATOR FOR THE DISPERSION OF POWDERS

A description of the design and purpose of this project is detailed in the 1992 THRU Annual Report (Wall et al., In Press). Completion of the generation system and subsystems was a priority during this calendar year. Calibration of a Dynacalibrator, including linear regressions and proper temperature settings for the unit, was achieved. Several standard bags were generated and analyzed in order to determine the high, medium, and low concentrations of acrolein to be used in combination with the Syloid 244 carrier. Numerous stainless steel and Syloid powder beds for the fluidized bed generator were prepared for use in the system. An aerosol photometer was used to monitor the degradation of the output of the aerosol generator. This gave the Research Engineering team an opportunity to further observe the responses from the bed generator.

Impactor and filter samples were taken in conjunction with the aerosol that was analyzed to develop a calibration of the aerosol analyzer. Exposures were performed at 2.5 and 5.0 ppm, with and without aerosol. A 10-ppm vapor-only exposure resulted in total mortality of the animals, and no additional experiments were performed at this level. Team members loaded and unloaded animals for each exposure and collected data pertinent to the study.

METHODS FOR ASSESSMENT OF COMBUSTION PRODUCT INSULT

A description of the design and purpose of this project is detailed in the 1992 THRU Annual Report (Wall et al., In Press). Efforts were directed toward supplying the furnace with 240 V single-phase electric power. It was also necessary to obtain a primary vacuum source and filtration equipment. Calibration of the orifice plate was also accomplished during this report period. Final phases of this project included calibration of the load cell and operation of the furnace at 400 °C. Extensive leak checking of the system occurred after installation of the system in Building 824.

SOLID FIRE EXTINGUISHANT AEROSOL CHARACTERIZATION SUPPORT

Assembly of a 690-L THRU chamber and a mist air scrubber was requested for the Army laboratory located in Building 824. Additionally, two electrically activated, pneumatically driven valves were acquired to be used in this inhalation system. A number of pieces of equipment, including a primary vacuum source, filtration subsystems, impactors, and aerosol analyzers were required to complete this system. Fabrication of primary and secondary plumbing and electrical systems for generating contaminant was completed. Several methods of contaminant generation were investigated before settling on the current system. Toxic Hazards Research Unit Research Engineering personnel were involved in the design and construction of the electronic generator used to produce the aerosol contaminant.

Extensive sealing of fittings was conducted to improve vacuum capabilities. Research Engineering participated in the initial trial runs of the system to locate and remove potential problems, develop basic protocols, test auxiliary equipment, and develop analytical techniques.

DESIGN AND CONSTRUCTION OF A THERMO GRAVIMETRIC ANALYZER SYSTEM

The THRU Research Engineering group was tasked with the fabrication of all systems and subsystems to support operation of a thermo gravimetric analyzer (TGA). Initial design of the system and its subsystems met the original specifications required by the government originator. Particular attention was paid to the main control panel, where the majority of operational functions would be performed (Figure 6.1-1). The layout of the panel and its assembly dictated the wiring of electronic equipment and the plumbing of valves, rotameters, and magnehelic gauges.

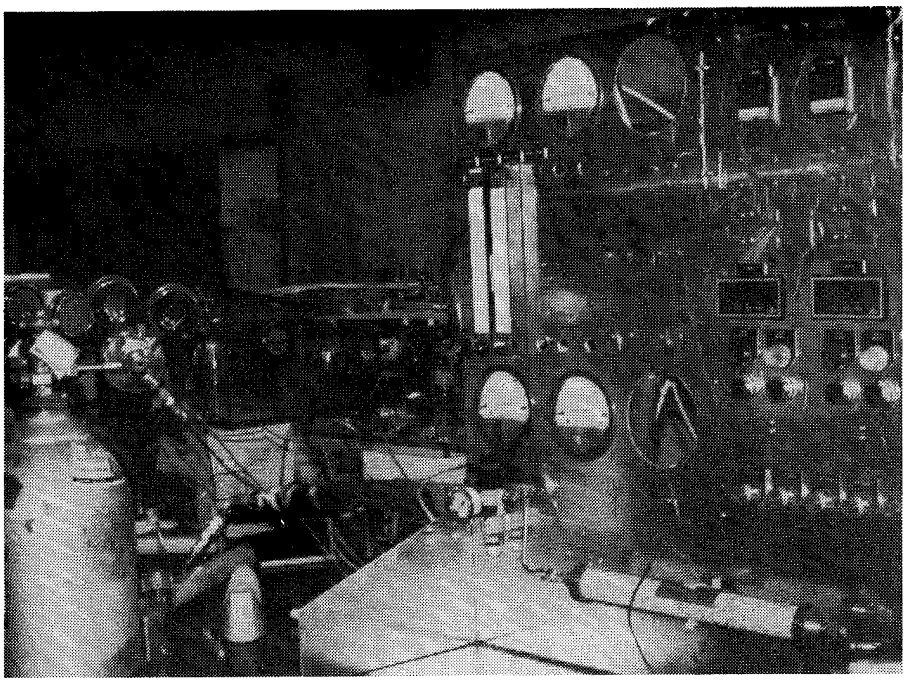


Figure 6.1-1. Thermo Gravimetric Analyzer System.

Custom-made manifolds were produced in order to support the flow requirements of the system. Specific magnehelic gauges and rotameters were obtained and installed. The rotameters were calibrated, and linear regressions were calculated and provided to the customer. The plumbing portion of the system was checked for leaks, and the electrical systems were approved. Final connections and last minute modifications were made to the TGA system.

TOXIC HAZARDS RESEARCH UNIT CHAMBER MAINTENANCE

Prior to use in a toluene inhalation study, a test of the integrity of Chambers 1 through 4 in Room 153, Building 79, from the input flow control valve to the exhaust valve, demonstrated the presence of some serious leaks.

Many of the shrink-fit connections on the 3-in. air lines, the compression fittings of the valves, and the connections of the glass sides of the chambers had become loose. The door-sealing closures also required adjustment. Using a small concentration of halothane within the chamber, a detector was used to locate the smallest leaks. The specificity of the detector allowed sealing with a silicon seal with no interference in locating other leaks.

The sealing process was effective in significantly increasing the time required for loss of chamber negative pressure. Chamber 1 went from less than 30 sec to over 11 min to drop from -3 to -2 in. of water, Chamber 2 went from less than 1 1/2 min to 11 min for a drop from -2 to -1 in. of water, and Chamber 3 took

30 min to drop from -5 to -4 in. after sealing. Chamber 4 was never satisfactorily sealed because of a metal fatigue crack. Therefore, Chamber 4 was used as a control chamber during the toluene inhalation study.

Following the sealing of the chambers, the input and outflow magnehelic pressure differentials were observed and compared with previous calibrations and their agreement across the individual chambers. The input and outflow orifice plates for Chamber 3 were removed, cleaned, and replaced. The Chambers 2 and 3 systems were then calibrated. Chamber 1 had been calibrated prior to the last study in that chamber. The slopes of the curves for all of the orifice plates approached 3 when plotting the square root of the pressure differential versus the calibrated flow. During use, Chamber 4 was operated with the same settings as the other three. The chamber flows were maintained at 6.6 cfm, providing more than 13 volume changes per hour. The chambers were operated at -0.5 to -1 in. of water.

The HyCal humidity gauges, Model CT 830 (HyCal Inst., El Monte, CA) were checked by comparing their readout with that of a sling psychrometer. Only one of six was found useful. This was placed in the control chamber to monitor the humidity during the toluene exposure. Calibration and repair of the others is currently in progress.

TOXIC HAZARDS RESEARCH UNIT RESEARCH SUPPORT STUDY REQUESTS

Design and Fabrication of a Fountain Cell Chamber

The design of a Teflon flow cell chamber was required for the optical measurement of flowing liquid streams. The cell chamber is to be used in fluorescence microscopy of live cells. The cell was designed with ports on both the inlet and the drain sides to enhance the fluid movement required. The flow had to be on a thin, even plane to provide consistent illumination and focus. The flow cell was fabricated to allow use of standard cover slips using threaded spacers to withstand pressure (Figure 6.1-2).

Universal Combustion Exposure Chamber/Plethysmograph

The THRU was requested to design and construct an apparatus to utilize whole-body plethysmography in conjunction with a low-volume, head-only, dynamic inhalation chamber. The design and construction would include interchangeable whole-body chambers to accommodate mice, rats, or guinea pigs. Each whole-body plethysmograph chamber has been precisely machined and has O-rings to secure it to the exposure chamber.

This chamber has been completed and has been accepted for use by the requestor (Figure 6.1-3). The completed chamber has a volume of approximately 2.6 L and has 3/4-in. quick disconnects on the intake and exhaust ends, as well as a removable baffle to allow for easy cleaning.

REFERENCES

Wall, H.G., D.E. Dodd, A. Vinegar, and M.G. Schneider, eds. In Press. *1992 Toxic Hazards Research Unit Annual Report.* AL/OE-TR-1993-0105, Wright-Patterson Air Force Base, OH: Armstrong Laboratory, and NMRI-93-33, Bethesda, MD: Naval Medical Research Institute (in press).

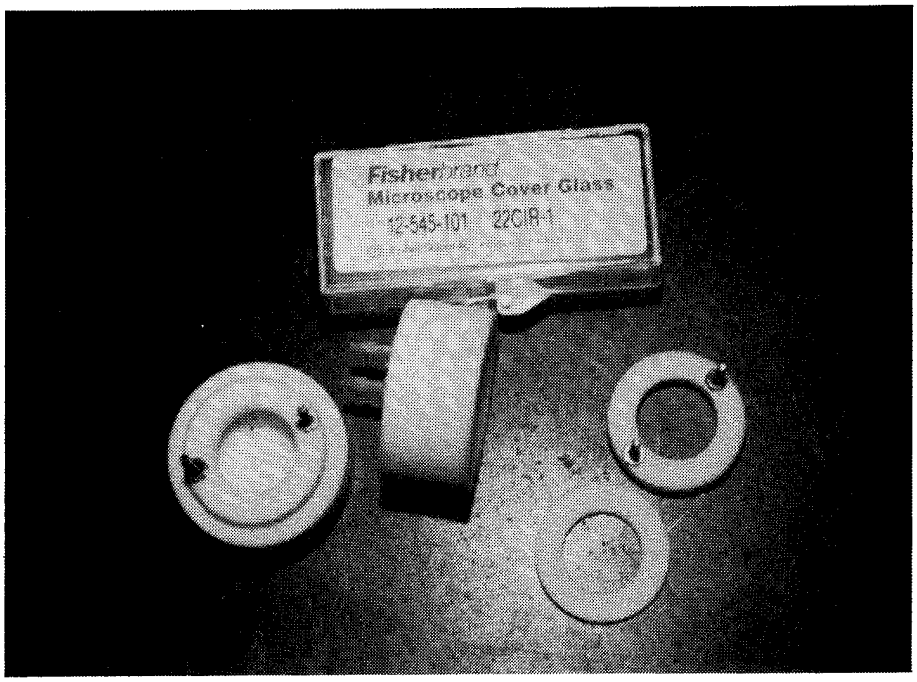


Figure 6.1-2. Fountain Cell Chamber.

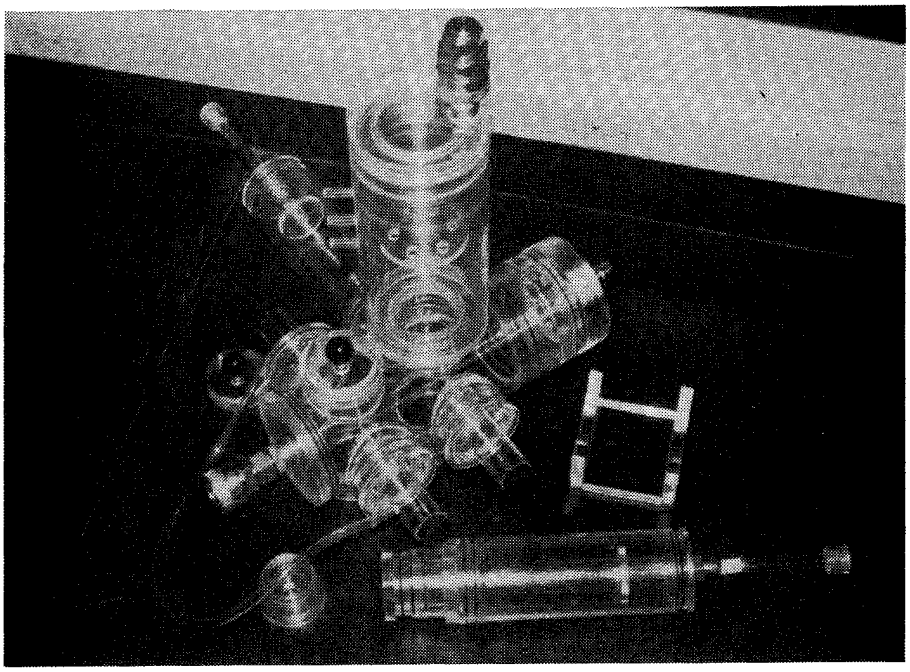


Figure 6.1-3. Universal Combustion Exposure Chamber/ Plethysmograph.

6.2 MATHEMATICS AND STATISTICS

C.D. Flemming

INTRODUCTION

This report covers projects in mathematics and statistics that were not assigned by study requests. This includes programming projects, statistical projects, and training and applied statistical research. All projects supported the research of the laboratory.

PROGRAMMING

Five RS/1 procedures were written and tested in support of the various modified Screening Information Data Set reproductive studies. The first procedure converts Pathtox extracted food or water consumption data into an RS/1 table. The second procedure adds columns to an existing table when the number of rows is larger than new table; hence, an existing table can be upgraded during a study, which saves time at the end of a study. The third procedure creates a table containing the day of experiment for predosing, premating, gestation, lactation, and postlactation. This table is used to make statistically usable tables for body weight, food or water consumption, dosage, and efficiency. The fourth procedure finds the dosage in milligrams per kilogram per day and the efficiency in percent. The fifth procedure converts the female food/water consumption, dosage, and efficiency tables to statistically useful tables.

An estrous cycle data entry RS/1 procedure was developed and tested. This program was used on two different studies. An RS/1 procedure was created to calculate the relative percent organ weight to body weight ratio. A second RS/1 procedure was developed to find the relative percent organ weight to brain ratio. The ratio of albumin to globulin is calculated by a new RS/1 procedure. The last three procedures are used on most of the toxicology studies that are conducted at this laboratory.

A FORTRAN program was developed and tested to do multivariate Monte Carlo simulation. The program assumed a normal or log-normal distribution. The program was developed to account for correlation among biological variables. The program will be used in connection with ACSL and PBPK_SIM to estimate chemical risk.

STATISTICS

Statistical analysis or consultation was done on the following research studies, which did not have an assigned THRU Study Number.

(1) Air Force study on the effect of perfluorodecanoic acid on the depression of carnitine in the plasma of guinea pigs

(2) Air Force study on the physiological properties of the skin of several different species and strains

(3) Air Force review of the pathology of several hydrazine studies

(4) Advisor to Air Force Institute of Technology master degree students

(5) Air Force study to compare hydraulic fluids

(6) Conducted several randomizations for several Air Force personnel

(7) Assisted several Air Force personnel on their study design

TRAINING AND APPLIED STATISTICAL RESEARCH

Toxic Hazards Research Unit (THRU) personnel assisted various laboratory personnel (contractor, Air Force, Navy, and Army) in the use of RS/1, SAS, and BMDP. On an ongoing basis, THRU personnel examined statistical and other literature to find new methods, or validate existing methods, for the handling of data.

6.3 PATHOLOGY SUPPORT (NECROPSY AND HISTOLOGY)

E.R. Kinkead

Necropsy support was provided in accordance with protocol requirements and standard operating procedures, or as determined by the veterinary pathologists. Routine, accepted methods of anesthesia were followed for terminal bleeding or euthanasia of laboratory animals. Necropsy procedures included determination of terminal body weights, detailed dissection, weighing of required organs, and collections and fixation of gross lesions and other required tissues for light microscopic examination.

Histologic processing of tissues included trimming, orientation of tissues in embedding cassettes, paraffin embedding, microtome sectioning of tissues to specific thickness, application of routine or special stain, and coverslipping. Uniformly prepared, high quality slides were prepared for review by veterinary pathologists. A pathology specimen archive is maintained with controlled access as required by Good Laboratory Practices.

The necropsy/histology technicians also were involved in organizing and collating path forms and slides from previously run hydrazine inhalation studies. Recuts were done on nasal sections for additional light microscopy evaluation. The necropsy technicians also were greatly involved in blood and tissue collections required for the methylene chloride and trichloroethylene studies.

The transmission electron microscopy laboratory has been restored to a functional working unit following completion of training by a histology technician. Cutting of both thick and thin sections of tissues is in progress in support of the acrolein nose-only inhalation study. Many more tissues await available time for processing. An experienced, full-time electron microscopist will be hired to oversee these and future research needs.

Necropsy/histology staff members processed the following research animals during this reporting period (Tables 6.3-1 and -2).

TABLE 6.3-1. TOTAL NUMBER OF ANIMALS LISTED BY SPECIES AND RESULTING NUMBER OF SLIDES

Species	Animals	Slides
Rat	1,418	10,767
Mouse	835	596
Baboon	5	35
Rabbit	5	25
Monkey	4	16
Swine	4	14
Guinea Pig	61	114
Dog	4	18
Human (Skin Samples)	1	1

TABLE 6.3-2. NUMBER OF ANIMALS PROCESSED BY MONTH AND RESULTING NUMBER OF SLIDES

Month	Number of Animals (in Species)		Number of Slides (In Type)	
1992:				
October	46	Mice	6	Mouse
	250	Rats	659	Rat
	1	Pig	1	Pig
	43	Guinea pigs	4	Guinea pig
	2	Baboons	14	Baboon
November	148	Mice	30	Mouse
	120	Rats	1017	Rat
	16	Guinea pigs	2	Guinea pig
December	145	Mice	2	Mouse
	102	Rats	62	Rat
	1	Guinea pig	14	Guinea pig
	1	Baboon	4	Baboon
	1	Dog	2	Dog
	2	Pigs	8	Pig
1993:				
January	50	Rats	284	Rat
	11	Mice	101	Mouse
	—	—	85	Guinea pig
February	198	Rats	387	Rat
	157	Mice	24	Mouse
	—	—	9	Guinea pig
	1	Baboon	12	Baboon
March	61	Rats	1294	Rat
	1	Guinea pig	—	—
	2	Dogs	4	Dog
	1	Baboon	21	Baboon
April	40	Mice	25	Mouse
	295	Rats	295	Rat
May	26	Mice	43	Mouse
	156	Rats	948	Rat

TABLE 6.3-2. NUMBER OF ANIMALS PROCESSED BY MONTH AND RESULTING NUMBER OF SLIDES

Month	Number of Animals (in Species)		Number of Slides (In Type)	
1993 (Continued)				
June	83	Mice	146	Mouse
	91	Rats	1336	Rat
	1	Dog	10	Dog
July	86	Mice	191	Mouse
	128	Rats	934	Rat
	5	Rabbits	25	Rabbit
August	87	Mice	9	Mouse
	234	Rats	1003	Rat
September	6	Mice	15	Mouse
	33	Rats	2048	Rat
	1	Pig	—	—

6.4 COMPUTER/ELECTRONICS SUPPORT

J.S. Stokes

The Computer and Electronics section of Research Support provided support for the Armstrong Laboratory/Toxicology Division VAX minicomputer, and for personal computer (PC), data acquisition and telecommunications systems hardware and software utilized by Toxic Hazards Research Unit (THRU) and Toxicology Division personnel. The following itemization illustrates a few of the services provided during this reporting period by Computer and Electronics Support.

- Wrote a dBASE III program to assist the AL/OET Hazardous Waste Coordinator with hazardous waste turn-in.

- Instructed the THRU's Property Management Custodian in the use of the property management program, which was written in dBASE III.

- Provided telephone and computer support services in the relocation of THRU personnel and computer systems, as follows.

Building 29:	To:
S. Godfrey	Building 429, Room 3B
M. Schneider	Building 79, Room 154E
AutoCAD system	Building 79T, Room 6
Building 79:	
H. Barton	Room 147B to Room 154C
W. Brashear	Room 147F to Room 125
D. Dodd	Room 154F to Room 154D
P. Fleenor	Room 154C to Room 154F
Building 79B:	
D. Courson	Building 79T, Room 2
W. Malcomb	Building 79T, Room 4
Building 79T:	
M. Ketcha	Building 79, Room 185
D. Mahle	Building 79, Room 185
W. Malcomb	Room 4 to Room 5
G. Randall	Building 79, Room 126A
S. Salins	Building 79, Room 150
R. Wolfe	Building 79, Room 149

Building 429:

C. Flemming	Building 79, Room 147D
ToxInfo system	Building 79, Room 147C

- Maintained stock of PC supply items for THRU computer users.

- Attended the PathTox Users Meeting in April. Presentations ranged from new PathTox capabilities to areas such as software validation.

- Extended the THRU's Lantastic local area network (LAN) printer sharing system to include Room 154D, which provides laser and dot matrix printing capabilities for all THRU front office personnel.

- Provided support and training for the THRU's Precision Measurement Equipment Laboratory custodian.

- Provided audio/visual and computer support for the 1993 Annual Toxicology Conference held at the Hope Conference Center (Study Request No. C04), as well as for the recent Trichloroethylene Workshop held at the Fairborn, OH, Holiday Inn Conference Center (Study Request No. F32).

- Worked with AL/OET Computer Support Personnel in planning and installing the Pathworks LAN in Buildings 79 and 824 for the Army. Installation of the LAN is continuing in Buildings 79, 838, and 824.

- Provided system management for Digital Equipment Corp. VAX computer in Building 79. Also provided support for users of Building 79 VAX computer.

- Wrote a VAX program (TEAMS) to allow Tri-Service e-mail to be sent to several functional groups of VAX users.

- Added capabilities of pH monitoring and control to rat liver perfusion data acquisition system hardware and software previously constructed for LCDR John Wyman at NMRI/TD in support of Study Request No. N10.

6.5 QUALITY ASSURANCE

M.G. Schneider

The Quality Assurance (QA) Coordinator attended national and local meetings of professional societies to extend knowledge regarding QA, Good Laboratory Practice (GLP) regulations, and records management. The QA Coordinator is a member of the Regulatory Review Committee of the Society of Quality Assurance(SQA). This Committee reviewed proposed regulations and prepared responses for the Society Board to present to regulatory agencies. The QA Coordinator participated in the activities of the Midwest Regional Chapter (MRC) of the SQA as Chair of the By-Laws Committee. The QA Coordinator attended seminars on ethics in QA and field study QA sponsored by the SQA in Atlanta, GA, and seminars on animal welfare act regulations, personal leadership, computer validation policy, subcontracting study phases, and GLP compliance sponsored by the MRC/SQA.

STANDARD OPERATING PROCEDURES ACTIVITIES

Forty-two new or revised standard operating procedures (SOPs) were reviewed and approved by the QA Unit (QAU) staff and were placed in the SOP manuals located in Toxic Hazards Research Unit (THRU) laboratory areas.

ARCHIVE ACTIVITIES

The QA Unit relocated the records archives from Building 29 to Building 429. This move was coordinated and conducted by the QA Unit staff. Processes such as records sorting and filing and report data audits have returned to normal. This new space did not allow for the installation of all of the archive equipment. The capacity of the archive system has been reached. Proposals to reestablish and expand the study records archives and install QA equipment in Building 429 have been made and are being evaluated.

Approximately 25,600 records were sorted and organized into file folders in preparation for microfilming. No records were sent for microfilming during this reporting period. Two copies of the microfilm were maintained at separate locations as security backup for the study records.

The QA Unit staff reviewed archived rat histopathology records from hydrazine studies conducted previously by the THRU for tumor incidences in support of Toxicology Division Pathology participation with the Hydrazine Coalition. The pathology tissue and slide archives also were accessed by the THRU's *In Vivo* Toxicology Group in support of this effort.

The QA Unit staff assumed oversight for the Test Substances and Pathology Tissues and Slides archives. This oversight includes ensuring that the study records contain accurate inventories of these archives. Actual maintenance and operation responsibilities for these archives remain with the Analytical Chemistry Section for the test substances and with the Histopathology Support Section for the pathology specimens.

AUDITS

The QA Coordinator conducted a systems audit at the request of ManTech Environmental QA management for the Health Effects Research program operated by ManTech Environmental for the U.S. Environmental Protection Agency at Research Triangle Park, NC. The ManTech Environmental QA Manager conducted a systems audit of the THRU. The findings of this audit produced a satisfactory rating for the THRU program.

The THRU managers, supervisors, and study directors participated in quarterly inspections of the use, maintenance, and data review practices for laboratory notebooks. At the end of this reporting period, 73 laboratory notebooks were assigned to THRU staff members.

The QA Unit reviewed listed protocols for GLP Standards (GLPS) and SOP compliance during this reporting period. Quality Assurance Coordinator approval was necessary prior to submission of the protocol for review and approval by the AL/OET Animal Care and Use Committee.

Study Request	**Protocol Title**
A02	Two-Generation Reproductive Study of 1,3,5-Trinitrobenzene
A03	Reproductive Toxicity Screen of LP1846 Formulation Administered in the Drinking Water of Sprague-Dawley Rats
F22	Acute Toxicity Evaluation of Ammonium Dinitramide
F25	Acute Toxicity Evaluation of Halon Replacement CF_3I
F29	Reproductive Toxicity Screen of Ammonium Dinitramide Administered in the Drinking Water of Sprague-Dawley Rats

The QA Unit conducted procedure and data audits during the course of the studies for the listed Study Requests during this reporting period.

Study Request	Test Phase
A01	-Range-finding study -Site visit by Qualtech, Inc. to review QA and study activities and records -Final study preparations review -Animal body weights, Group B Dose #4, and Group A Dose #5 -Sacrifice dams from Group A -Sacrifice dams from Group B
A02	Range-finding study: -Animal QC -Animal body weights -Randomization of animals -Initiate feeding study -Data and records -Diet assay records -Site visit by Qualtech, Inc. to review QA and study activities and records -Room 4, Building 838 temperature and relative humidity data -Sacrifice dams -Methemoglobin assay -Data and records -Room 4, Building 838 temperature and relative humidity data Modified Screening Information Data Set study: -Animal QC -Animal body weights -Feeder maintenance -Animal body weights -Food consumption -Initiate mating period -Sacrifice male rats -Day 0 postnatal procedures (weigh dams, count and sex pups) -Day 1 postnatal procedures (weigh pups) -Day 4 postnatal procedures (weigh dams and pups, cull pups) -Behavioral tests on female rats
A03	-Animal body weights -Animal randomization -Drinking water consumption -Initiation of drinking water study -Preparation of animal drinking water -Behavioral tests on female rats -Sacrifice male rats -Postnatal pup weights -Postnatal dam body weights -Sacrifice pups -Sacrifice dams -Sacrifice male rats

F08	-Inhalation exposure to HCFC-123 -Urine sample preparation for trifluoroacetic acid analysis
F09	-Skin-only exposure to dibromomethane
F22	-Dose oral toxicity rats with 5 g/kg ammonium dinitramide -Sacrifice dermal toxicity rabbits -Animal body weights for palatability study -Drinking water consumption -Initiation of drinking water palatability study -Sampling for ammonium dinitramide stability test -Sacrifice rats from palatability study -Dose acute oral toxicity rats -Sacrifice acute oral toxicity rats
F25	-Inhalation exposure at 0.5% CF_3I -Sacrifice rats 3 days postexposure -Sacrifice rats 14 days postexposure
F26	-Animal chamber acclimatization period -Inhalation exposure to toluene -Sacrifice of female rats
N03	-Sacrifice female rats at 5 weeks posttreatment -Sacrifice male rats at 9 weeks posttreatment -Sperm motility measurements -Sacrifice male rats at 18 weeks posttreatment -Sperm motility measurements -Sperm count
N09	-Inhalation exposure to Syloid/acrolein mixture -Sacrifice rats 4 h postexposure -Sacrifice rats 14 days postexposure
N10	-Blood pressure measurements -Cerebral blood flow measurements
N15	-Gas uptake study with methylene chloride

The QA Unit completed study report and data audits for the listed Study Requests during this reporting period.

Study Request	**Study Title**
F08	Metabolism of 2,2-Dichloro-1,1,1-trifluoroethane (HCFC-123) by Human Hepatic Microsomes

F11	Inhibition Kinetics for Metabolism of Vinyl Chloride/Trichloroethylene Mixtures: Pharmacokinetics Modeling Using *In Vivo* and *In Vitro* Studies with Rats
F13	Evaluation of Chloropentafluorobenzene as an Intake Simulant for Chemical Defense Training
F15	Lactational Transfer of Tetrachloroethylene in Rats
F18	Acute Toxicity of Quadricyclane
F26	Generation and Analysis of Toluene Exposures in THRU Chambers
N10	Vascular Toxicity Associated with Exposure to Propylene Glycol 1,2-Dinitrate

The QA Unit completed QA editing on reports submitted to the Contract Technical Monitor and on manuscripts submitted for publication in peer-review literature and journals.

Study Request	**Report Title**
F15	"Occupational" Exposure of Infants to Toxic Chemicals Via Breast Milk
F15	Lactation Transfer of Tetrachloroethylene in Rats
F18	Acute Toxicity of Quadricyclane
F20	Evaluation of QSAR for Use in Predictive Toxicology Modeling

6.6 HEALTH AND SAFETY

M.G. Schneider

The Toxic Hazards Research Unit (THRU) Health and Safety Representative (HSR) assumed occupational health and safety responsibilities for both THRU and Toxicology Division programs during this reporting period.

TRAINING

All THRU staff members who work with chemicals received a special 4-h training course in hazardous waste management and the U.S. Environmental Protection Agency Resource Conservation and Recovery Act (RCRA), conducted by the staff of Wright-Patterson Air Force Base (WPAFB) Environmental Management, and a 4-h training course in hazardous waste disposal practices, conducted by the Toxicology Division Hazardous Waste Coordinator.

Twelve staff members received training in cardiopulmonary resuscitation/first aid and achieved certification. W.J. Malcomb, of Research Engineering, provided this instruction.

The HSR attended training seminars concerning Occupational Safety and Health Administration (OSHA) inspections, conducted by the Ohio Business and Industry Conference; OSHA regulations update, conducted by the Dayton Area Chamber of Commerce; RCRA changes for 1993, conducted by WPAFB Environmental Management; and the Air Force environmental impact analysis process, conducted by Shipley Associates.

CHEMICAL SAFETY

The chemical inventories for the THRU, the Toxicology Division, and the Army Occupational Health Research Detachment were combined and placed on the VAX computer system as a searchable text file for staff reference in determining the availability of chemicals before ordering. Chemical spill plans located at each THRU work area were reviewed and updated. The chemical inventories posted for each of the THRU work areas were revised monthly. A program was initiated to identify excess and out-of-date chemicals for turn-in and disposal. Vendors who had supplied materials for which Material Safety Data Sheets (MSDSs) were not on file were contacted to provide the necessary items. This included requests for MSDSs for materials no longer in use in the laboratory. A common chemical storage area was established in Room 117, Building 79 to minimize storage within the research laboratories.

PERSONNEL SAFETY

Programs to provide the THRU staff with proper personal protective equipment were continued. Vendors were identified and selected through competitive bidding to provide safety glasses; safety shoes; and labwear such as scrubs, shop uniforms, and labcoats. Staff members were provided with and trained in the use of respiratory protection as part of the new-employee orientation in health and safety.

The medical surveillance program was reviewed for suitability of employee testing conducted and results reporting. This program included preemployment, annual, and exit physical examinations and testing, as well as emergency care for on-the-job injury, follow-up evaluations and Workers Compensation paperwork initiation. The program also provided consulting for employees concerned with occupationally related illness and job-related issues for pregnancy. Thirty-six annual and 11 exit physical examinations were scheduled during this reporting period. Ten employees with risk for bloodbourne pathogen exposure were provided immunization to hepatitis B. Three employees received consultations with the medical surveillance provider relating to job issues.

Seven incidents resulting in injury/illness to staff occurred, with 24.5 lost work days and 21 days with work restrictions. Three of the seven incidents were reportable on the OSHA 200 log. Worker's Compensation claims were initiated for two of these incidents.

SAFETY COMMITTEE

The THRU has an established Safety Committee with membership consisting of two representatives from each Program, a representative from Research Support, and the HSR, who chairs the Committee. This Committee met monthly. The Committee continued its method of safety inspections with total inspections each month. Results were reported and reviewed at each monthly meeting. Minutes of the meeting were distributed to THRU management for information and action purposes. Health and Safety Awards were presented by the Safety Committee to D.A. Mahle, Simulation Research and Development, for individual safety contribution, and to the staff of Necropsy Support, M.A. Parish and J.W. Nicholson, for group safety effort.

INSPECTIONS

The THRU work areas were subject to quarterly safety inspections by AL/OET safety staff members, accompanied by the HSR. These inspections noted continued improvement in several areas, especially housekeeping. Response to comments and concerns was always timely.

7.1 1993 CONFERENCE ON THE RISK ASSESSMENT PARADIGM AFTER TEN YEARS: SCIENCE, POLICY, AND PRACTICE, THEN, NOW, AND IN THE FUTURE

H.A. Barton, D.R. Mattie[1], and W.B. Peirano[2]

The Conference on The Risk Assessment Paradigm After Ten Years: Science, Policy, and Practice Then, Now, and in the Future was held at the Hope Hotel and Conference Center, Wright-Patterson Air Force Base, OH, from 5 to 8 April 1993. The conference was sponsored by the Toxicology Division, Occupational and Environmental Health Directorate, Armstrong Laboratory; the Naval Medical Research Institute/Toxicology Detachment; the Army Biomedical Research and Development Laboratory; and the U.S. Environmental Criteria and Assessment Office, U.S. Environmental Protection Agency; with the cooperation of the National Research Council Committee on Toxicology.

The goals of the conference were to promote exchange of information between those who develop risk assessment methodologies and those who perform risk assessment. Specifically

- to acquaint individuals with the basics of the risk assessment process as originally developed 10 years ago by the National Academy of Sciences;
- to highlight current issues in risk assessment, emphasizing the strengths and weaknesses of the current paradigm and areas needing change; and
- to present and discuss current research related to improving the risk assessment process.

The conference featured invited presentations by noted individuals in the field of risk assessment and a poster session on issues relevant to the conference theme.

The rapid increase in the utilization of risk assessment, since the presentation by the National Academy of Science in 1983 of an analytical paradigm, has raised numerous and difficult scientific and policy issues. The Basics of Risk Assessment (Session I) reviewed the use of paradigm. This was followed by consideration of Case Comparisons — Issues/Lessons Learned (Session II), and suggestions for Where the Paradigm Needs Change (Session III).

---

[1]Toxicology Division, Armstrong Laboratory, Wright-Patterson Air Force Base, OH.

[2]Environmental Criteria and Assessment Office, Environmental Protection Agency, Cincinnati, OH

The speakers provided insights gained as risk assessment has moved from relatively simple default approaches toward addressing the complexities of exposure scenarios and chemical toxicities. This move toward complexity and flexibility represents a natural progression for the field. Risk assessors often started by screening for those situations that might be of concern by using health-protective assumptions and simplifications. Realities are inevitably different than the simplified case, so additional information and methodologies to utilize it must be developed and implemented. This presents a challenge to researchers, practitioners, and users due to their significantly different time frames, technical backgrounds, and policy perspectives. Risk assessment must improve to assist in decision making despite the limitations of available information and human understanding.

The next two sessions of the conference described Advancing the Science of Risk Assessment (Sessions IV and V). Several speakers discussed scientific advances that have implications for risk assessment, particularly of carcinogens. As our knowledge of cancer processes improves, it reinforces the fact that there are several diseases we refer to collectively as cancer. Any single quantitative risk assessment methodology shows its weaknesses when it tries to address all of these identically. Other speakers addressed issues associated with mathematical modeling for exposure routes, carcinogenicity data, or noncarcinogenic effects.

Risk assessment has thrived in this age of computerization. Early risk assessments used simplified assumptions and approaches due, in part, to limitations of access to computational power. The speakers in Sessions IV and V described how risk assessment can gain from increasing modeling sophistication. They also discussed indications that mathematical methods can move us a only short way in the absence of either concrete information (e.g., mechanisms of toxicity) or clear policy decisions (e.g., how to protect various populations).

Much of the conference focused on how new scientific information and improved methodologies are increasing the sophistication of risk assessment, but the final session addressed the challenges of Risk Communication (Session VI). Mathematical modeling, Monte Carlo simulation, pharmacokinetics, and mechanisms of toxicity must be pathways to greater clarity and conciseness, not just complexity and technical sophistication.

The public arena in which risk assessment exists is one of human concerns and desires for an improved life. It is this context that makes risk assessment such a challenging multidisciplinary field. The presentations from this conference demonstrated that developing a flow from information gathering to analysis to discussion to decision is still a challenge, even within the risk assessment/management/communication fields. The languages of mathematics and human speech must come together to facilitate discussion and decision making if risk assessment and risk management are to achieve the success that society desires from them.

7.2 1994 CONFERENCE ON TEMPORAL ASPECTS IN RISK ASSESSMENT FOR NONCANCER END POINTS

D.E. Dodd

Planning was initiated in August 1993 for the 1994 toxicology conference "Temporal Aspects in Risk Assessment for NonCancer Endpoints." It will be held 18 through 20 April 1994 at the Hope Hotel and Conference Center at Wright-Patterson Air Force Base, OH. The conference is being sponsored by the Toxicology Division, Occupational and Environmental Health Directorate, Armstrong Laboratory; the Naval Medical Research Institute Detachment (Toxicology); the U.S. Army Medical Research Detachment of Walter Reed Army Institute of Research; the Office of Research and Development, U.S. Environmental Protection Agency; and the Division of Toxicology, Agency for Toxic Substances and Disease Registry. The planning committee for the conference includes representatives from each sponsoring agency and the Toxic Hazards Research Unit (THRU).

The goals of the conference are to

- recognize temporal issues in the risk assessment process;
- examine critical time factors in hazard identification, dose response, and exposure characterization; and
- explore limitations in risk characterization.

The THRU's work plan, which designated a conference coordinator and described invitation and registration procedures, technical support, the administration of continuing education credits, and publication procedures, was submitted to and approved by the Contract Technical Monitor in October 1992. The planning committee has selected the journal *Inhalation Toxicology* as the source for peer-review publication of the conference proceedings. Initial conference announcements were placed in several major scientific journals and were mailed to over 2000 individuals.

The conference will feature invited presentations by noted individuals in the field of toxicology and other disciplines relevant to toxicological risk assessment, a poster session on topics germane to the conference theme, and a database/modeling preview to allow presentation and demonstration of existing databases to interested attendees. The proposed agenda includes five platform sessions.

Session I	Introductory Session
Session II	Temporal Factors of Exposure in Identifying Hazards
Session III	Effects of Exposure Patterns on Dose/Response
Session IV	Exposure Assessment and the Life Cycle Timeline
Session V	Characterization of Risk in a Temporal Context

APPENDIX A

TOXIC HAZARDS RESEARCH UNIT PERSONNEL
AS OF 30 SEPTEMBER 1993

Darol E. Dodd, Ph.D., DABT
LABORATORY DIRECTOR

RESEARCH SUPPORT
Patricia M. Fleenor
Supervisor

Staff:
Angell, Mary Ann
Brooks, Shelia D.
Stokes, James S

QUALITY ASSURANCE/ HEALTH AND SAFETY
Mathias G. Schneider, Jr.
Coordinator

Staff:
Godfrey, Susan M.

ADMINISTRATION
Lois A. Doncaster
Supervisor

RISK ASSESSMENT
Hugh A. Barton, Ph.D.
Supervisor

Staff:
Flemming, Carlyle D.

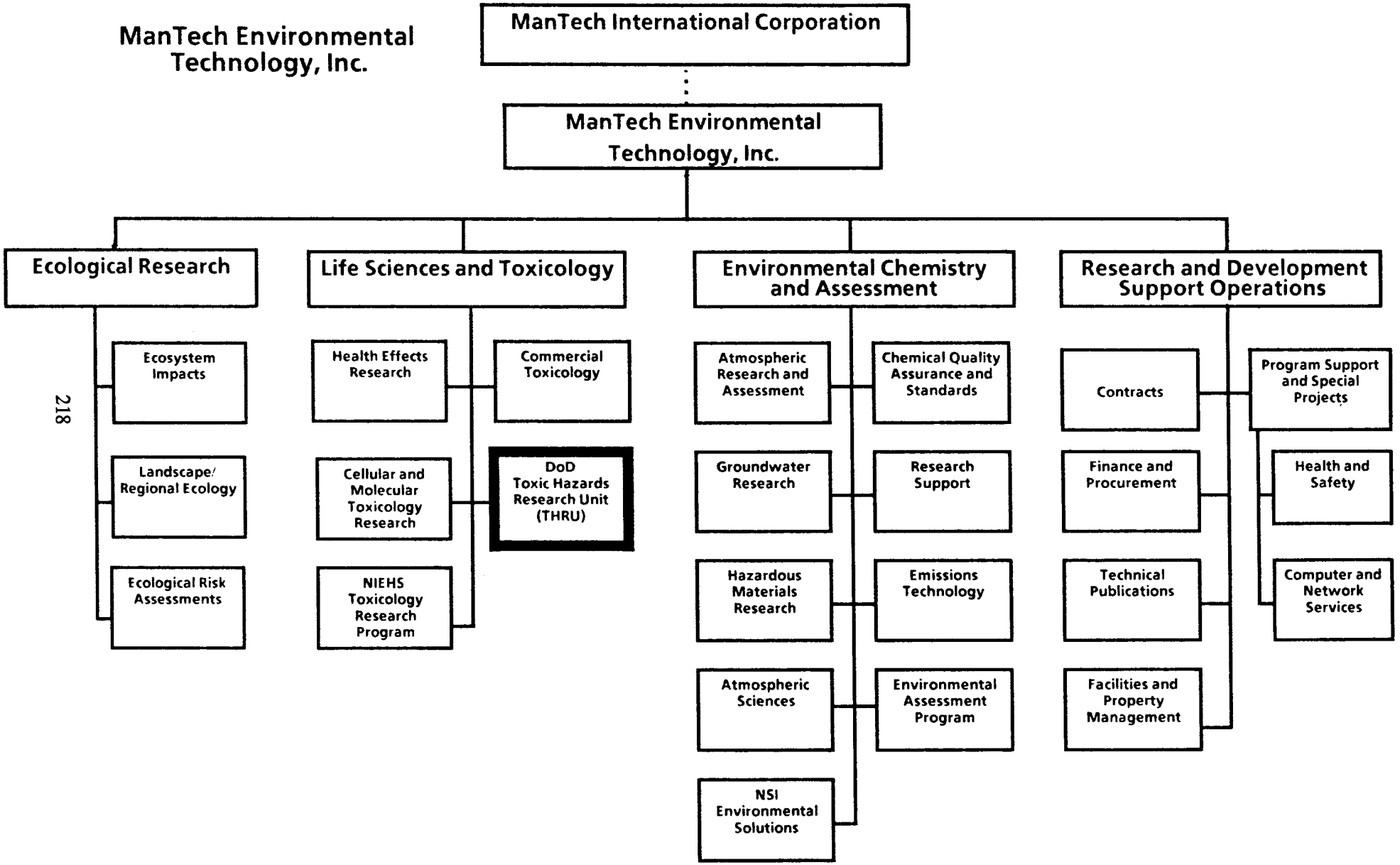
TOXICOLOGY
Darol E. Dodd, Ph.D., DABT
Manager

Staff:
Courson, David L.
Drerup, Joanne M.
Godfrey, Richard J.
Kinkead, Edwin R.
Leahy, Harold F.
Malcomb, Willie J.
Neely, Gloria A.
Nicholson, Jerry W.
Parish, Margaret A.
Salins, Stephanie A.
Sonntag, William B.
Wolfe, Robin E.

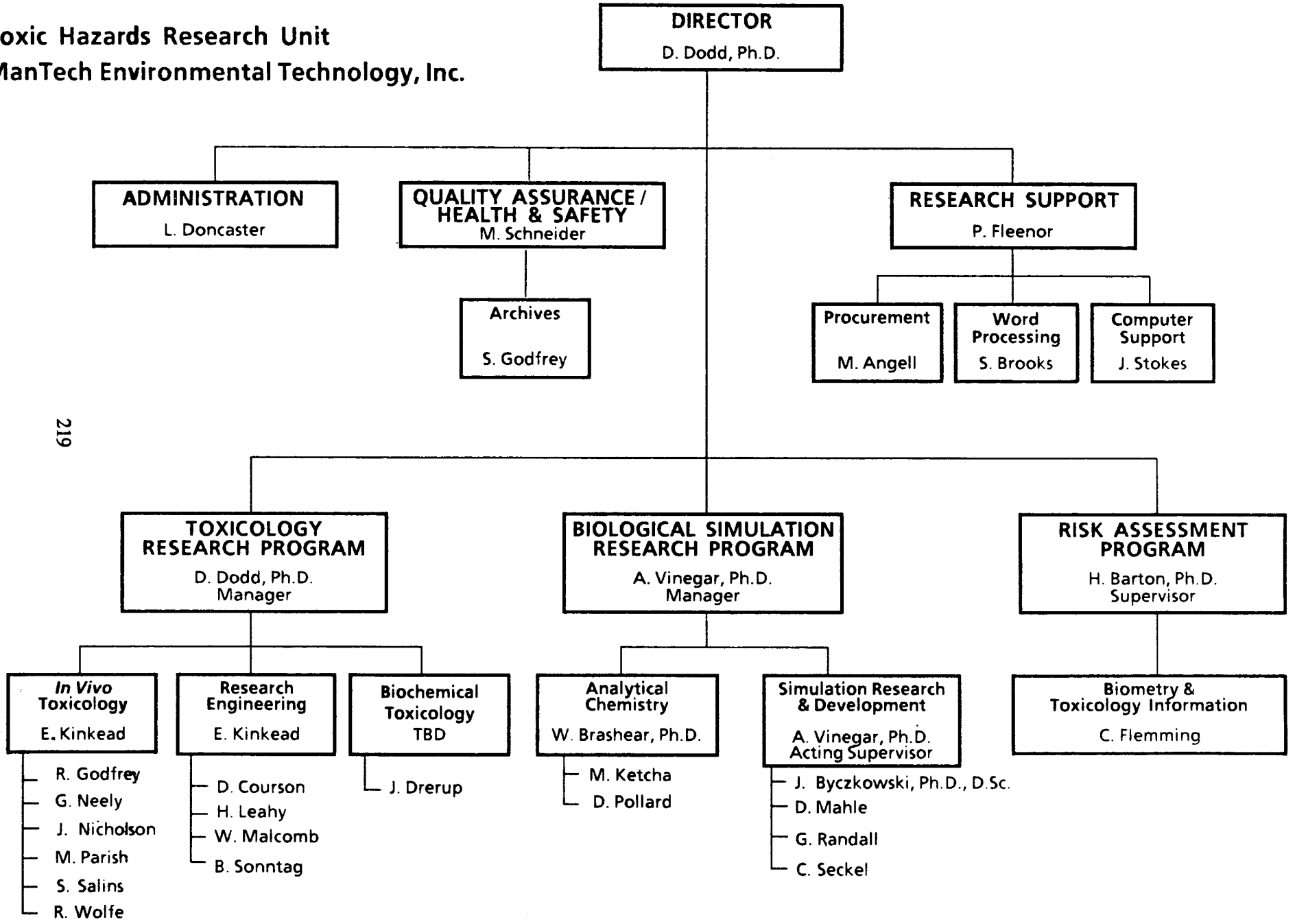
BIOLOGICAL SIMULATION
Allen Vinegar, Ph.D.
Manager

Staff:
Brashear, Wayne T., Ph.D.
Byczkowski, Janusz Z. Ph.D., D. Sc.
Ketcha, Marcia M.
Mahle, Deirdre A.
Pollard, Daniel L.
Randall, Gia M.
Seckel, Constance S.

ManTech Environmental Technology, Inc.



Toxic Hazards Research Unit
ManTech Environmental Technology, Inc.



APPENDIX B

SUBMITTED JOURNAL PUBLICATIONS, TECHNICAL REPORTS, AND LETTER REPORTS

JOURNAL PUBLICATIONS

Andersen, M.E., **H.J. Clewell III, D.A. Mahle, and J.M. Gearhart**. Submitted. Gas uptake studies of the deuterium isotope effects on dichloromethane metabolism in female B6C3F1 mice *in vivo*. *Toxicol. Appl. Pharmacol.*

Barton, H.A. and M.A. Marletta. In Press. Comparison of aniline hydroxylation by hemoglobin and microsomal cytochrome P450 using stable isotopes. *Toxicol. Lett.*

Barton, H.A., D.R. Mattie, and W.B. Peirano, eds. 1994. Proceedings of the 1993 Conference on The Risk Assessment Paradigm After Ten Years: Policy and Practice Then, Now, and in the Future. *Risk Analysis* 14(3):217-378.

Bruner, R.H., **E.R. Kinkead**, T.P. O'Neill, **C.D. Flemming**, D.R. Mattie, C.A. Russell, and **H.G. Wall**. 1993. The toxicologic and oncogenic potential of JP-4 jet fuel vapors in rats and mice: 12-Month intermittent exposures. *Fundam. Appl. Toxicol.* 20:97-110.

Byczkowski, J.Z., E.R. Kinkead, H.F. Leahy, G.M. Randall, and J.W. Fisher. In Press. Computer simulation of lactational transfer of tetrachloroethylene in rats using a physiologically based model. *Toxicol. Appl. Pharmacol.*

Byczkowski, J.Z. 1993. Lipid peroxidation and benzo[*a*]pyrene cooxygenation. Abstract. *Nutrition* 9:89.

Byczkowski, J.Z., J.M. Gearhart, and J.W. Fisher. 1994. "Occupational" exposure of infants to toxic chemicals via breast milk. *Nutrition* 10(1):43-48.

Byczkowski, J.Z. and J.W. Fisher. 1993. Lactational transfer of tetrachloroethylene in rats. Conference on the Risk Assessment Paradigm After Ten Years, Wright-Patterson AFB, OH, Biographies and Papers EPA/630/R-93-039.

Byczkowski, J.Z. and J.W. Fisher. 1994. Lactational transfer of tetrachloroethylene in rats. *Risk Analysis* 14(3):339-349.

Byczkowski, J.Z., P.J. Ramgoolie, and A.P. Kulkarni. 1992. Some aspects of activation and inhibition of rat brain lipoxygenase. *Int. J. Biochem.* 24:1691-1695.

Byczkowski, J.Z. 1993. Lipid peroxidation and benzo[*a*]pyrene cooxygenation. In: T. Yasugi, H. Nakamura, and M. Soma, eds. *Advances in Polyunsaturated Fatty Acid Research*, pp. 107-110. Amsterdam: Exerpta Medica.

Clewell III, H.J. 1993. Coupling of computer modeling with *in vitro* methodologies to reduce animal usage in toxicity testing. *Toxicol. Lett.* 68(1,2):101-118.

Dodd, D.E., W.T. Brashear, and A. Vinegar. 1993. Metabolism and pharmacokinetics of selected Halon replacement candidates. *Toxicol. Lett.* 68:37-47.

Gearhart, J.M., D.A. Mahle, R.J. Greene, **C.S. Seckel, C.D. Flemming**, J.W. Fisher, and **H.J. Clewell III.** 1993. Variability of physiologically based pharmacokinetic (PBPK) model parameters and their effects on PBPK model predictions in a risk assessment for perchloroethylene. *Toxicol. Lett.* 68(1,2):131-144.

Gearhart, J.M., C.S. Seckel, and A. Vinegar. 1993. *In vivo* metabolism of chloroform in B6C3F$_1$ mice determined by the method of gas uptake: The effects of body temperature on tissue partition coefficients and metabolism. *Toxicol. Applied Pharmacol.* 119:256-266.

Godin, C.S., E.C. Kimmel, **J.M. Drerup, H.F. Leahy, and D.L. Pollard**. 1993. Effect of exposure route on measurement of blood pressure by tail cuff in F-344 rats exposed to OTTO Fuel II. *Toxicol. Lett.* 66:147-155.

Godin, C.S., J. He, J.M. Drerup, and J.F. Wyman. Accepted. Assessment of biomarkers of vascular toxicity associated with exposure to propylene glycol 1,2-dinitrate. *Toxicol. Lett.*

Godin, C.S., M.M. Ketcha, J.M. Drerup, and A. Vinegar. Submitted. Metabolism of 2,2-dichloro-1,1,1-trifluoroethane (HCFC-123) by human hepatic microsomes. *Drug Metabol. Dispos.*

Godin, C.S., J.M. Drerup, and A. Vinegar. 1993. Conditions influencing the rat liver microsomal metabolism of 2,2,-dichloro-1,1,1-trifluoroethane (HCFC-123). *Drug Metab. Dispos.* 21(3):551-553.

Jepson, G.W., D.K. Hoover, R.K. Black, J.D. McCafferty, **D.A. Mahle, and J.M. Gearhart**. Accepted. A partition coefficient determination method for non-volatile and intermediate volatility chemicals in biological tissues. *Fundam. Appl. Toxicol.*

Kinkead, E.R., R.E. Wolfe, S.A. Salins, C.S. Godin, P.P. Lu, M.M. Ketcha, A. Thilager, and **W.T. Brashear**. 1993. *N*-methyl-*n'*-nitroguanidine: Irritation, sensitization, and acute oral toxicity, genotoxicity, and methods for analysis in biological samples. *Toxicol. Ind. Health* 9(3):457-477.

Kinkead, E.R., R.E. Wolfe, and S.A. Salins. 1993. Acute irritation and sensitization potential of shale-derived JP-5 jet fuel. *Acute Toxicity Data J.* 11(6):705.

Kinkead, E.R., R.E. Wolfe, and S.A. Salins. 1993. Acute irritation and sensitization potential of JP-7 jet fuel. *Acute Toxicity Data J.* 11(6):707.

Kinkead, E.R., S.A. Salins, and R.E. Wolfe. 1993. Acute irritation and sensitization potential of shale-derived JP-4 jet fuel. *Acute Toxicity Data J.* 11(6):702.

Kinkead, E.R., R.E. Wolfe, and S.A. Salins. 1993. Acute irritation and sensitization potential of petroleum-derived JP-5 jet fuel. *Acute Toxicity Data J.* 11(6):706.

Kinkead, E.R., R.E. Wolfe, and S.A. Salins. 1993. Acute irritation and sensitization potential of shale-derived diesel marine fuel. *Acute Toxicity Data J.* 11(6):704.

Kinkead, E.R., R.E. Wolfe, and S.A. Salins. 1993. Acute irritation and sensitization potential of petroleum-derived diesel marine fuel. *Acute Toxicity Data J.* 11(6):703.

Kinkead, E.R. and R.E. Wolfe. 1993. Dermal toxicity of various compounds to female rabbits. *Acute Toxicity Data J.* 11(6):712.

Kinkead, E.R., S.A. Salins, and R.E. Wolfe. 1993. Acute irritation and sensitization potential of petroleum-derived JP-4 jet fuel. *Acute Toxicity Data J.* 11(6):699.

Kinkead, E.R., S.A. Salins, and R.E. Wolfe. 1993. Acute irritation and sensitization potential of JP-TS jet fuel. *Acute Toxicity Data J.* 11(6):701.

Kinkead, E.R., S.A. Salins, and R.E. Wolfe. 1993. Acute irritation and sensitization potential of JP-8 jet fuel. *Acute Toxicity Data J.* 11(6):700.

Kinkead, E.R. and R.E. Wolfe. 1993. Single oral toxicity of various organic compounds. *Acute Toxicity Data J.* 11(6):713.

Kulkarni, A.P. and **J.Z. Byczkowski**. 1993. Hepatotoxicity. In: E. Hodgson and P.E. Levi, eds. *Introduction to Biochemical Toxicology*, 20:459-490. Norwalk, CT: Appleton and Lange.

Marit, G.B., M.E. George, **D.E. Dodd, and A. Vinegar**. Submitted. Hepatoxocity in guinea pigs following acute inhalation exposure to HCFC-123. *Toxicol. Pathol.*

Mattie, D.R., **D.E. Dodd, and H.J. Clewell III**, eds. 1993. Applications of advances in toxicology to risk assessment. Proceedings of the 1992 Toxicology Conference, Wright-Patterson AFB, OH. *Toxicol. Lett.* 68(1,2):1-266.

McDougal, J.N. and **D.E. Dodd**. 1993. Air Force approach to risk assessment for Halon replacements. *Toxicol. Lett.* 68:31-35.

TECHNICAL REPORTS

Brashear, W.T. and P.P. Lu. 1993. Evaluation of QSAR for use in predictive toxicology modeling. AL/OE-TR-1993-0113. Wright-Patterson AFB, OH: Armstrong Laboratory, Toxicology Division.

Brashear, W.T., R.J. Greene, and D.L. Pollard. 1992. Storage stability of pCTFE carboxylic acid metabolites. AL-TR-1992-0143. Wright-Patterson AFB, OH: Armstrong Laboratory.

Clewell, H.J., III, ed. 1993. Proceedings of the 1991 Conference on Chemical Risk Assessment in the DoD: Science, Policy, and Practice. AL-TR-1993-0001 and NMRI-93-10. Wright-Patterson AFB, OH: Armstrong Laboratory, and Naval Medical Research Institute Detachment/Toxicology.

Clewell, H.J., III and B.M. Jarnot 1993. Evaluation of chloropentafluorobenzene as an intake simulant for chemical defense training. AL-TR-1993-0002. Wright-Patterson AFB, OH: Armstrong Laboratory, Toxicology Division.

Dodd, D.E., H.J. Clewell III, and D.R. Mattie, eds. 1993. Proceedings of the 1992 Conference on Toxicology: Applications of Advances in Toxicology to Risk Assessment. AL/OE-TR-1993-0059 and NMRI-93-18. Wright-Patterson AFB, OH: Armstrong Laboratory, and Naval Medical Research Institute Detachment/Toxicology.

Gearhart, J.M., C.S. Seckel, and A. Vinegar. 1993. *In vivo* metabolism of chloroform in B6C3F$_1$ mice determined by the method of gas uptake: The effects of body temperature on tissue partition coefficients and metabolism. AL-TR-1993-0114. Wright-Patterson AFB, OH: Armstrong Laboratory, Toxicology Division.

Godin, C.S., E.C. Kimmel, **J.M. Drerup, H.F. Leahy, and D.L. Pollard**. 1992. Effect of exposure route on measurement of blood pressure by tail cuff in F-344 rats exposed to OTTO Fuel II. AL-TR-1992-0150. Wright-Patterson AFB, OH: Armstrong Laboratory, Toxicology Division.

Godin, C.S., and H.G. Wall. 1992. Structure-activity comparison of hydrazine to other nasotoxic chemicals. AL-TR-1992-0116. Wright-Patterson AFB, OH: Armstrong Laboratory.

Kinkead, E.R., R.E. Wolfe, C.D. Flemming, G.B. Marit, M.L. Barth. 1992. Acute delayed neurotoxicity evaluation of two jet engine oils using a modified Navy and EPA protocol. AL-TR-1992-0115, NMRI-92-74. Wright-Patterson AFB, OH: Armstrong Laboratory, Toxicology Division, and Naval Medical Research Institute Detachment/Toxicology.

Kinkead, E.R., S.A. Salins, and R.E. Wolfe. 1993. Acute toxicity of quadricyclane. AL-TR-1993-0046. Wright-Patterson AFB, OH: Armstrong Laboratory, Toxicology Division.

Wall, H.G., D.E. Dodd, A. Vinegar, and M.G. Schneider, eds. 1993. *1992 Toxic Hazards Research Unit Annual Report*. AL/OE-TR-1993-0105, NMRI-93-33. Wright-Patterson Air Force Base, OH: Armstrong Laboratory, Toxicology Division, and Naval Medical Research Institute Detachment/Toxicology.

Williams, R.J., **A. Vinegar, J.Z. Byczkowski**, J.R. Creech, R.K. Black, S.K. Neurath, and G.W. Jepson. In Press. Gas uptake kinetics of bromotrifluoromethane (Halon 1301) and its proposed replacement iodotrifluoromethane (CF_3I). Wright-Patterson Air Force Base, OH: Armstrong Laboratory, Toxicology Division.

LETTER REPORTS

Clewell III, H.J., J.M. Gearhart, and M.E. Andersen. 1993. Analysis of the metabolism of methylene chloride in the B6C3F$_1$ mouse and its implications for human carcinogenic risk. Final report to OSHA Division of Consumer Affairs, Washington, DC.

Kinkead, E.R., S.A. Salins, R.J. Godfrey, and H.F. Leahy. 1993. Generation and analysis of toluene exposures in THRU chambers. Letter report. Wright-Patterson AFB, OH: Armstrong Laboratory, Toxicology Division.

APPENDIX C

PRESENTATIONS AT SCIENTIFIC MEETINGS

Bankston, L.A., **J.M. Gearhart, R.J. Greene**, S. Fortunatio, C. Bryant, and J.W. Fisher. 1993. Partition Coefficient Determination for Mixtures of Volatile Chemicals in Human Breast Milk and Blood. Poster presentation, Society of Toxicology Annual Meeting, New Orleans, LA. Abstract. *The Toxicologist* 13(1):174.

Bankston, L.A., **J.M. Gearhart, R.J. Greene**, S. Fortunatio, C. Bryant, and J.W. Fisher. 1993. Partition Coefficient Determination for Mixtures of Volatile Chemicals in Human Breast Milk and Blood. Poster presentation, Conference on the Risk Assessment Paradigm after Ten Years, Wright-Patterson AFB, OH.

Barth, M.E., **E.R. Kinkead**, J.T. Yang, and C.R. Mackerer. 1993. A Comparison of Protocols to Assess Neurotoxicity of Formulations Containing Phosphate Esters. Poster presentation, Society of Toxicology Annual Meeting, New Orleans, LA. Abstract. *The Toxicologist* 13(1):122.

Barton, H.A. 1993. Metabolism of Chemical Mixtures: Vinyl Chloride/Trichloroethylene Physiologically Based Pharmacokinetic (PBPK) Modeling. Presented at Wright State University, Fairborn, OH (cosponsored by Wright State University Department of Pharmacology/Toxicology and WPAFB Toxicology Group), February.

Barton, H.A., J.R. Creech, **C.S. Seckel, and C.S. Godin**. 1993. Mixtures of Chlorinated Ethylenes: Physiologically Based Pharmacokinetic (PBPK) Modeling. Poster presentation, Society of Toxicology Annual Meeting, New Orleans, LA. Abstract. *The Toxicologist* 13(1):357.

Barton, H.A., C.S. Seckel, J.R. Creech, and **C.S. Godin**. 1993. Inhibition Kinetics for Metabolism of Chlorinated Ethylene Mixtures: Pharmacokinetic Modeling. Poster presentation, Midwest Cytochromes P450 Symposium, Purdue University, Lafayette, IN, September.

Barton, H.A. 1993. Evaluating Low Level Acute Toxicity to Humans from Airborne Chemicals: The Interplay of Toxicology, Air Modeling, and Public Health. Poster presentation, Conference on the Risk Assessment Paradigm After Ten Years, Wright-Patterson AFB, OH, April.

Byczkowski, J.Z., E.R. Kinkead, R.J. Greene, L.A. Bankston, and J.W. Fisher. 1993. Physiologically Based Modeling of the Lactational Transfer of Tetrachloroethylene. Poster presentation, 11th Annual Spring Meeting of the Ohio Valley Society of Toxicology, Cincinnati, OH, June.

Byczkowski, J.Z., E.R. Kinkead, R.J. Greene, L.A. Bankston, and J.W. Fisher. 1993. Physiologically Based Modeling of the Lactational Transfer of Tetrachloroethylene. Poster presentation, Society of Toxicology Annual Meeting, New Orleans, LA. Abstract. *The Toxicologist* 13(1):354.

Byczkowski, J.Z. 1993. Lactational Transfer of Tetrachloroethylene to Infant: Computer Simulations and Predictions Using Physiologically Based Pharmacokinetic Model. Poster presentation, Conference on the Risk Assessment Paradigm after Ten Years, Wright-Patterson AFB, OH, April.

Byczkowski, J.Z. 1993. Lactational Transfer of Tetrachloroethylene to Infant. Presentation to Department of Pharmacology/Toxicology, Wright State University, Fairborn, OH, May.

Byczkowski, J.Z. 1993. Lactational Transfer of Tetrachloroethylene to Infant: Computer Simulations and Predictions Using Physiologically Based Pharmacokinetic Model. Presented to the Department of Animal Physiology, Gdansk, Poland, August.

Byczkowski, J.Z. and J.W. Fisher. 1993. Quantitative Approach to Assess Risk from Lactational Transfer of Tetrachloroethylene to Breast-Fed Infants. Program and Abstracts E-10, Society for Risk Analysis Annual Meeting, Savannah, GA, December.

Byczkowski, J.Z. 1993. Lipid Peroxidation and Benzo[*a*]pyrene Cooxygenation. Presentation to Department of Toxicology, Medical Academy of Gdansk, Poland, July.

Creech, J.R., **C.S. Seckel, and H.A. Barton**. 1993. Inhalation Uptake and Metabolism of Vinyl Chloride (VC) and Trichloroethylene (TCE) Mixtures. Poster presentation, Conference on the Risk Assessment Paradigm after Ten Years, Wright-Patterson AFB, OH, April.

Creech, J.R. and **H.A. Barton**. 1993. Inhalation Uptake and Metabolism of Vinyl Chloride (VC) and Trichloroethylene (TCE) Mixtures. Poster presentation, Society of Toxicology Annual Meeting, New Orleans, LA. Abstract. *The Toxicologist* 13(1):436.

Eurell, T.E., D.R. Mattie, V.S. Dunovant, G.B. Marit, and **C.D. Flemming**. 1993. Hematology and Blood Chemistry Values for Fisher 344 and NCI-Black Reiter Male Rats After Exposure to Decalin and JP-8. Poster presentation, Society of Toxicology Annual Meeting, New Orleans, LA. Abstract. *The Toxicologist* 13(1):356.

Fisher, J.W., **J.M. Gearhart, R.J. Greene,** L.A. Bankston, C. Bryant, and S.J. Fortunato. 1993. Estimating the Lactational Transfer of Volatile Chemicals in Women Using a Physiological Model. Poster presentation, Society of Toxicology Annual Meeting, New Orleans, LA. Abstract. *The Toxicologist* 13(1):356.

Flemming, C.D., H.A. Barton, J.M. Gearhart, O.M. Little, R.L. Carpenter, and **H.J. Clewell III**. 1993. The Effect of Body Fat and Body Weight from Navy Subpopulations on Dose Metrics Used in Risk Assessment. Poster presentation, Conference on the Risk Assessment Paradigm after Ten Years, Wright-Patterson AFB, OH, April.

Gearhart, J.M., H.J. Clewell III, and M.E. Andersen. 1993. Identification of Metabolic Parameters in the B6C3F_1 Mouse for Use in a Pharmacokinetic Risk Assessment for Methylene Chloride. Poster presentation, Society of Toxicology 1994 Annual Meeting, Dallas, TX (Abstract).

Gearhart, J.M., H.J. Clewell III, **D.A. Mahle,** and M.E. Andersen. 1993. Mechanism of Substrate Oxidation by Cytochrome P450 2E1 Inferred from Deuterium Isotope Effects on Dichloromethane Metabolism *In Vivo*. Presentation to International Society for the Study of Xenobiotics, Tucson, AZ, October.

Gearhart, J.M., D.A. Mahle, C.S. Seckel, and C.D. Flemming. 1993. Physiologically Based Simulation of Perchloroethylene (PCE) Pharmacokinetics in Humans. Poster presentation, Conference on the Risk Assessment Paradigm After Ten Years, Wright-Patterson AFB, OH, April.

Gearhart, J.M., D.A. Mahle, C.S. Seckel, and C.D. Flemming. 1993. Physiologically Based Simulation of Perchloroethylene (PCE) Pharmacokinetics in Humans. Poster presentation, Society of Toxicology Annual Meeting, New Orleans, LA. Abstract. *The Toxicologist* 13(1):356.

Godin, C.S. 1993. The Structure-Activity of Hydrazine and Its Relationship to Other Nasotoxic Chemicals. Presentation to Hydrazine Coalition of the Chemical Manufacturers Association, Dayton, OH, January.

Godin, C.S., J.M. Drerup, and A. Vinegar. 1993. Influence of Oxygen Concentration and Enzyme Induction on Metabolism of HCFC-123 *In Vitro*. Poster presentation, Conference on the Risk Assessment Paradigm After Ten Years, Wright-Patterson AFB, OH, April.

Godin, C.S., J.M. Drerup, and A. Vinegar. Influence of Oxygen Concentration and Enzyme Induction on Metabolism of HCFC-123 *in vitro*. Poster presentation, Society of Toxicology Annual Meeting, New Orleans, LA. Abstract. *The Toxicologist* 13, 1:387, 1993.

Greene, R.J., J.M. Gearhart, L.A. Bankston, D.J. Finch, C. Bryant, S.J. Fortunato, and J.W. Fisher. 1993. Effects of Protein and Lipid Content on Human Breast Milk/Air Partitions of Selected Chemicals. Poster presentation, Society of Toxicology Annual Meeting, New Orleans, LA. Abstract. *The Toxicologist* 13(1):130.

Kinkead, E.R., R.E. Wolfe, C.D. Flemming, G.B. Marit, M.L. Barth. 1993. Acute Delayed Neurotoxicity Evaluation of Two Jet Engine Oils Using a Modified Navy and EPA Protocol. Poster presentation, Society of Toxicology Annual Meeting, New Orleans, LA. Abstract. *The Toxicologist* 13(1):122.

Kinkead, E.R., R.E. Wolfe, C.D. Flemming, G.B. Marit, M.L. Barth. 1993. Acute Delayed Neurotoxicity Evaluation of Two Jet Engine Oils Using a Modified Navy and EPA Protocol. Poster presentation, Conference on the Risk Assessment Paradigm after Ten Years, Wright-Patterson AFB, OH, April.

Kinkead, E.R., R.E. Wolfe, S.A. Salins, C. Miller, and **J.R. Latendresse.** 1993. Single-Generation Reproduction study of 1,3,5-Trinitrobenzene in the Diet of Sprague-Dawley Rats. Poster presentation, Society of Toxicology 1994 Annual Meeting, Dallas, TX (Abstract).

Lee, D.J., R.D. Dunmire, F.G. Mappin, M.M. Crabbe, C.L. Hadick, **J.R. Latendresse, and C.D. Flemming.** 1993. Blood Patch Pleurodesis in a Rabbit Model. Presented to the Society of Air Force Clinical Surgeons, February.

Lu, P.P., W.T. Brashear, E.R. Kinkead, and D.L. Pollard. 1993. Chromatographic Analysis for the Toxic Metabolite of *N*-methyl-*n*'-nitroguanidine in Biological Samples. Poster presentation, PITTCON '93, Atlanta, GA, March.

Mahle, D.A., R.J. Greene, J.M. Gearhart, and J.W. Fisher. 1993. Metabolism of Perchloroethylene and the Disposition of the Metabolites Trichloroacetate and Dichloroacetate. Poster presentation, Conference on the Risk Assessment Paradigm After Ten Years, Wright-Patterson AFB, OH, April.

Mahle, D.A., R.J. Greene, J.W. Fisher, and **J.M. Gearhart**. 1993. Metabolism of Perchloroethylene and the Disposition of the Metabolites Trichloroacetate and Dichloroacetate. Poster presentation, Society of Toxicology Annual Meeting, New Orleans, LA. Abstract. *The Toxicologist* 13(1):174.

McDougal, J.N., **D.E. Dodd**, and S.R. Skaggs. 1993. Recommending Exposure Limits for Halon Replacements. Presented to Halon Alternatives Technical Working Conference, Albuquerque, NM, April.

McDougal, J.N. and **D.E. Dodd**. 1993. Air Force Rationale for Recommending an EEGL for the Halon 1211 Replacement HCFC-123. Presented to the Committee on Toxicology, Aspen, CO, June.

Salins, S.A., R.E. Wolfe, and E.R. Kinkead. 1993. Acute Toxicity Evaluation of Quadricyclane. Poster presentation, American Industrial Hygiene Conference, New Orleans, LA, May.

Salins, S.A., R.E. Wolfe, E.R. Kinkead, and D.R. Tocco. 1993. Acute and Subacute Toxicity Evaluation of Ammonium Dinitramide. 1994 American Industrial Hygiene Conference, Anaheim, CA (Abstract).

Schneider, M.G. 1993. Building an Effective Training Program. Presented at Society of Quality Assurance Annual Meeting, San Francisco, CA, October.

Seckel, C.S., H.G. Wall, E.R. Kinkead, D.R. Mattie, **D.L. Pollard, and A. Vinegar**. 1993. Comparison of Liver Changes Following Administration of Polychlorotrifluoroethylene (PCTFE) in Rhesus Monkeys with Those Seen in Rats. Poster presentation, Society of Toxicology Annual Meeting, New Orleans, LA. Abstract. *The Toxicologist* 13(1):340.

Vinegar, A., C.S. Seckel, C.S. Godin, D.L. Pollard, M.M. Ketcha, and E.R. Kinkead. 1993. Enzyme Inhibitory Activity Affects the Kinetics and Metabolism of 2,2-Dichloro-1,1,1-trifluoroethane (HCFC-123) *In Vivo*. Poster presentation, Society of Toxicology Annual Meeting, New Orleans, LA. Abstract. *The Toxicologist* 13(1):359.

Vinegar, A., R.J. Williams, J.N. McDougal, and J.W. Fisher. 1993. Pharmacokinetics of 2,2-Dichloro-1,1,1-trifluoroethane (HCFC-123) and Its Metabolite Trifluoroacetic Acid (TFA): Development of a Physiologically Based Pharmacokinetic Model. Poster presentation, International Society for the Study of Xenobiotics, Tucson, AZ, October.

Wolfe, R.E., S.A. Salins, E.R. Kinkead, D.J. Caldwell, C.R. Miller, and J.R. Latendresse. 1993. A Reproductive Toxicity Screen of a Liquid Propellent (LP) Formulation Administered in the Drinking Water of Sprague-Dawley Rats. Poster presentation, 1994 American Industrial Hygiene Conference, Anaheim, CA (Abstract).

Wolfe, R.E., S.A. Salins, J.R. Latendresse, and E.R. Kinkead. 1993. Repeated-Dose Gavage Studies with H-19457C Hydraulic Fluid. Poster presentation, American Industrial Hygiene Conference, New Orleans, LA, May.

APPENDIX D

TOXIC HAZARDS RESEARCH UNIT GUEST SPEAKERS

Date	Title	Presenter
19 Jul 93	Carcinogen Risk Assessment — Opening the Black Box	E. Wattenberg
21 Jul 93	Interspecies Comparisons of Tissue Disposition of Perchloroethylene Using a PBPK Model	X. Chen

U. S. Government Printing Office 1995 650-075/00130